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ANNUAL RESEARCH PROGRESS REPORT

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number) This report describes progress in		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) During Fiscal Years 1976 & 77 progress was obtained at the Letterman Army Institute of research in the following research areas: basic and applied research in skin diseases of military importance; the effects of hemorrhagic shock on the heart and brain; basic and applied nutritional biochemistry; basic biochemical processes of metabolism; basic and applied nutrition; clinical nutrition; the metabolism of normal and as altered by disease; the evaluation of insect repellent; serodiagnosis of leishmaniasis; the determination of exposure thresholds of coherent (see reverse)		

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→ radiation producing damage to the eye and skin; basic and applied studies on blood and blood products storage; work performance on man and military dogs; and research computer science. The progress made in ~~these~~ fiscal years is described in the reports of the work units presented.

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(1976 and 1977)

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FOREWORD

The research conducted at the Letterman Army Institute of Research, Presidio of San Francisco, California, was accomplished in Fiscal Years 1976 & 1977 under the following projects and task areas:

3A161101A91C - In-House Laboratory Independent Research

- 01 - Biomedical Sciences
- 03 - Human Ecology

3A161102B71R - Research in Biomedical Sciences

- 02 - Internal Medicine
- 03 - Psychiatry

3A162110A821 - Combat Surgery

3A762768A824 - Radiation Injury and Protection

- 03 - Lasers

3A762759A831 - Other Tropical Medicine

3A762760A822 - Military Internal Medicine

- 01 - Internal Medicine
- 02 - Nutrition and Wholesomeness Support for DOD Food Program

3A762760A837 - Military Animal Resources Development

Tasks are subdivided into work units, as appropriate, to accomplish the objects of the task.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

Note - Important

All information that is available indicates that the DD Form 1498 was never intended to be a financial document. However, various

Foreword (Cont)

individuals and agencies have tried to utilize the 1498s as financial records. The assumption has been made that summing the dollar values contained on the 1498s within previous Annual Progress Reports should provide the total amount of money expended by the Activity for the preceding year. Normally, there are expenditures that would not be reflected by the 1498s--particularly expenditures under the funding category "Special Purpose Equipment." In FY 76 & 77, there were other large expenditures that are not contained in the 1498s present in this report. These funds were expended against "start-up" FIC numbers to permit establishment of individual divisions and/or departments at LAIR. The explanation and breakout of the total expenditure of funds for these "start-up" functions is extremely lengthy and will be excluded from this report. If any agency or individual has need to have access to this information, it can be obtained by writing to:

Commander
Letterman Army Institute of Research
ATTN: Chief, Program & Accounting
Presidio of San Francisco, CA 94129

or: HQDA (SGRD-SSC)
WASH DC 20314

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3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISB'N INSTR'N	9. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61101A	3A161101A91C	00	040			
B. CONTRIBUTING							
C. COORDINATING	None						
11. TITLE (Precede with Security Classification Code)*							
(U) The Molecular Basis of Vitamin A Activity (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
72 07		CONT		DA		C. In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE		EXPIRATION		PRECEDING		20	
B. NUMBER* Not Applicable				FISCAL		0.3	
C. TYPE		D. AMOUNT		CURRENT		8	
E. KIND OF AWARD		F. CUM. AMT.		77		1.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS*				NAME* Letterman Army Institute of Research Biochemistry Division Department of Nutrition Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME* Sauberlich, H.E., DAC TELEPHONE 415-561-4323 SOCIAL SECURITY ACCOUNT NUMBER			
RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415-561-3600				ASSOCIATE INVESTIGATORS NAME: Bashor, M.M., DAC NAME:			
21. GENERAL USE				POC:DA			
Foreign Intelligence Not Applicable							
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Vitamins; (U) Vitamin A; (U) Retinol; (U) Military Nutrition; (U) Vitamin A Metabolism; (U) Vitamin A Functions; (U) Military Medicine							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number precede text of each with Security Classification Code.)							
<p>23. (U) The function of vitamin A, aside from its participation in vision remains unknown. Yet the effects of a deficiency of the vitamin are marked and diverse. Of military interest, is its apparent participation in resistance, infection and stress and its requirements in wound healing. Vitamin A is also responsible for normal keratinization in epithelial tissues. In order to provide a better rationale for the use of vitamin A and its requirements, investigations would be conducted on vitamin A functions at the molecular level. The studies would determine the specific tissue functions of vitamin A and its active form.</p> <p>24. (U) Studies will be conducted on the involvement of vitamin A on sulfur amino acid metabolism and sulfation, on membrane carrier lipids, on erythrocytes and membrane functions, on wound healing and infection, on amino acid incorporation and enzyme synthesis, and on DNA and RNA metabolism. Studies will use tissue culture techniques, cellular blocking agents, labeled substrates, electronmicroscopy, etc.</p> <p>25. (U) 75 07 - 76 09 The retention of radioisotope-labeled vitamin A during processing for electron microscopy was investigated using the livers and kidneys from vitamin A deficient rats. The procedure developed permitted 96-98% retention of the label in the tissues (Plopper, C.G., Wallace, D.L., and Pietzsch, J.D., <u>Stain Technol.</u> 50:339, 1975). Results of experiments on the autoradiographic localization of ³H-vitamin A in the rat adrenal gland have been evaluated and published (Wallace, D. L., Plopper, C.G., Bucci, T.J., and Sauberlich, H.E., <u>Life Sci.</u>, 17:1693-1698, 1975).</p>							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

U.S. GPO: 1974-540-843/8691

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	040	The Molecular Basis of Vitamin A Activity

The following investigation has been conducted under this Work Unit:

STUDY NO. 1 Investigation of the Subcellular Distribution of Vitamin A in the Rat

Retention of radioisotope-labeled vitamin A was investigated in the hepatic and renal tissue of vitamin A deficient rats during the process of preparing the specimens for electron microscopic examination. Of the procedures studied, water-soluble embedding retained the highest percentage of label in the tissue (96-98%). Inclusion of osmium tetroxide in the processing sequence and minimal exposure of tissue to lipid solvents were necessary for good retention of labeled vitamin A in tissues. Cellular distribution of vitamin A in the rat adrenal gland was evaluated by autoradiography. Vitamin A was concentrated in the lipid droplets of epithelial cells of the zona fasciculata and zona reticularis. A small amount of vitamin A was also present in the cytoplasm and nucleus of these cells. The zona glomerulosa contained little vitamin A either in lipid droplets or in the remainder of the cell. The medulla had essentially no vitamin A. In similar autoradiography studies conducted on the kidney, the majority of the vitamin A was present in the cells of the proximal convoluted tubules. A small number of endothelial cells in the medulla and interstitial cells in the cortex were also labeled. Sensitive fluorometric procedures for the measurement of vitamin A in biological samples are being investigated. Of military interest is the apparent participation of vitamin A in resistance, infection, stress, and wound healing. The investigations were initiated to elucidate vitamin A functions at the molecular level in order to provide a better rationale for the use of the vitamin in nutrition, health, and disease.

BODY OF REPORT

WORK UNIT NO. 040

The Molecular Basis of Vitamin A Activity

STUDY NO. 1

Investigation of the Subcellular Distribution of Vitamin A in the Rat

PROBLEM:

The function of vitamin A, aside from its participation in vision remains unknown. Yet the effects of a deficiency of the vitamin are marked and diverse. Of military interest is its apparent participation in resistance, infection, and stress and its requirements in wound healing. The initial studies were designed to investigate, by autoradiography, the localization of vitamin A in rat tissues and, thereby, obtain information to serve as a basis for further studies on the molecular functions of vitamin A.

RESULTS AND DISCUSSION OF RESULTS:

In preliminary studies, the retention of radioisotope-labeled vitamin A was investigated in the livers and kidneys from vitamin A deficient rats during processing of tissue for electron microscopic studies. Retinol-15- ^{14}C (3 $\mu\text{Ci}/\text{animal}$) was administered by esophageal intubation to male rats which had been maintained on a vitamin A deficient diet for five or six weeks postweaning. Eight hours after administration of the label, the animals were sacrificed and the tissues processed. Of the processing procedures studied, water-soluble embedding retained the highest percentage of label in the tissue (liver: 96.3%; kidney: 98.7%). Inclusion of osmium tetroxide in the processing sequence and minimal exposure of tissue to lipid solvents were necessary for good retention of labeled vitamin A in tissues.

Results of experiments on the autoradiographic localization of ^3H -vitamin A in rat adrenal glands and kidneys have been evaluated and published. The studies revealed that for the adrenal gland, vitamin A was concentrated in the lipid droplets of epithelial cells of the zona fasciculata and zona reticularis. A small amount of vitamin A was also present in the cytoplasm and nucleus of these cells. The zona glomerulosa contained very little vitamin A either in lipid droplets or in the remainder of the cell. The medulla had essentially no vitamin A.

The autoradiography studies conducted on the kidney revealed that most of the vitamin A was present in cells of the proximal convoluted tubules. An extremely small number of endothelial cells in the medulla and interstitial cells in the cortex were also labeled.

Vitamin A appears to be filtered by the glomerulus and resorbed by tubular epithelium which may permit the re-circulation and/or conversion of retinol to retinoic acid. The presence of vitamin A in certain interstitial and endothelial cells is unexplained.

To provide improved techniques for investigating vitamin A functions, sensitive fluorometric procedures for measuring vitamin A are under evaluation. The fluorometric method under study permits a 100-fold increase in sensitivity over currently used assay procedures. The availability of a sensitive and reliable micro-assay procedure will enhance the use of tissue culture systems and small animal models in investigating vitamin A functions and interactions. The recent report of abnormal proteins appearing in the urine of the vitamin A deficient rat has been confirmed at the Letterman Army Institute of Research.

CONCLUSIONS:

1. A suitable procedure has been developed for the retention of vitamin A during the processing of tissues for electron microscopy.
2. Cellular distribution of vitamin A in the rat adrenal gland strongly supports the hypothesis that vitamin A is involved in the synthesis of glucocorticoid hormones.
3. The role of the kidney in vitamin A metabolism remains unclear. Vitamin A appears to be filtered by the glomerulus and resorbed by tubular epithelium which may permit the re-circulation and/or conversion of retinol to retinoic acid.

RECOMMENDATIONS:

The development of a highly sensitive micromethod for measuring retinol is essential for application to tissue culture systems and animal models used in studies on vitamin A functions and metabolism.

PUBLICATIONS:

1. Plopper, C.G., D.L. Wallace and J.D. Pietzsch. Extraction of ¹⁴C-vitamin A from rat liver and kidney during processing for transmission electron microscopy. Stain Technol., 50:339, 1975.
2. Wallace, D.L., C.G. Plopper, T.J. Bucci and H.E. Sauberlich. Autoradiographic localization of vitamin A in the adrenal gland of rats. Life Sci., 17:1693, 1975.
3. Plopper, C.G., D.L. Wallace, T.J. Bucci and H.E. Sauberlich. Autoradiographic localization of vitamin A in the kidney of rats. Proc. Soc. Exptl. Biol. Med. (In press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OA 6378	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTR'M	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUMMARY
76 04 02	H Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER		
A. PRIMARY	61101A	3A161101A91C	00		041		
XXXXXXXXXX							
XXXXXXXXXX	None						
11. TITLE (Precede with Security Classification Code)*							
(U) Wholesomeness Aspects of Military Subsistence (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
006500 Food, 007800 Hyg. & Sanitation, 010100 Microbiology							
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A. DATES/EFFECTIVE:				PRECEDING		76	
B. NUMBER* Not Applicable				FISCAL YEAR		77	
C. TYPE:				CURRENCY		0.4	
D. KIND OF AWARD:						0	
E. AMOUNT:						0	
F. CUM. AMT.						0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME* Letterman Army Institute of Research Food Hygiene Division			
ADDRESS*				ADDRESS* Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME* Fowler, James L., COL, VC			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-2963			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Fruin, John T., LTC, VC			
				NAME: POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Public Health; (U) Food Poisoning; (U) Microbiology; (U) Bacteriological Techniques; (U) Food Microbiological Data Bank							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Food safety in military food preparation and handling operations is of great importance. The Department of Defense must necessarily employ mass feeding techniques to feed large numbers of personnel. Due to inherent characteristics in the procedures necessary to prepare and serve large numbers of personnel, foodborne illness outbreaks are more likely to occur in such facilities than in smaller operations. A thorough knowledge of microbiological physiology, microbiological procedures, and incidence of foodborne pathogens is basic in food hygiene and in preventing foodborne illnesses.</p> <p>24. (U) An on-going study entitled "Investigation of Selective Media and Confirmatory Techniques for <i>Bacillus cereus</i>" will be transferred to another work unit. Comparisons of the characteristics of two selective mediums recommended for the enumeration of <i>B. cereus</i> from foods have been completed, and a limited survey of ground beef for presence of the organism is being conducted. Techniques for producing a purified spore suspension have been developed and utilized, and procedures for fractionating and collecting spore components are under study. If feasible, a diagnostic antisera will be produced in rabbits for definitive identification of <i>B. cereus</i> isolates from food products.</p> <p>25. (U) 75 07 - 76 09 The KG medium of Kim and Goepfert has, in our hands, proven to be quite satisfactory for the isolation and enumeration of <i>B. cereus</i> from food products. It is recommended that this medium be adopted by Army Area Laboratories for this purpose. The results of the survey of ground beef for the presence of <i>B. cereus</i> is presently being tabulated, and a purified spore suspension is available for fractionation. At this time we are investigating suitable fractionation procedures, and pilot production of <i>B. cereus</i> antisera in rabbits should be accomplished in the near future. This work unit is being terminated. Certain aspects will be continued under Work Unit 004: Military Food Hygiene.</p>							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	041	Wholesomeness Aspects of Military Subsistence

The following investigation has been conducted under this work unit in the current fiscal year.

STUDY NO. 4 Investigation of Selective Media and Confirmatory Techniques for *Bacillus cereus*

STUDY NO. 4 The Department of Defense (DoD) has unique responsibilities for food safety in its food service operations in several ways. In addition to the necessity of providing food service to military personnel throughout the world, DoD has the responsibility for procurement, inspection, analyses, shipment, storage, and issue of its food items. The responsibility for adherence to proper food preparation and handling procedures lies with DoD personnel; likewise, military public health officials are responsible for food safety. In military operations, a foodborne illness outbreak could be disastrous.

Bacillus cereus has been recognized for many years as a causative agent of foodborne illness in continental Europe and for a relatively short time in the United States. At the present time, there is no clear-cut information of the extent of contamination of food items with this organism, although a few surveys have been accomplished. Part of the lack of information may be due to the lack of generally accepted microbiological methodology for the isolation and enumeration of *B. cereus*. Two recently developed selective media - the KG medium of Kim and Goepfert and the MYP medium of Mossel et al. show promise of being suitable for use in furthering the knowledge of *B. cereus*' role in foodborne illness.

A comparison of the characteristics of the two media has been made, and a limited survey of the incidence of *B. cereus* in ground beef has begun. Initial steps in the development of *B. cereus* antisera for definitive identification of the organism have been made.

BODY OF REPORT

WORK UNIT NO. 041

Wholesomeness Aspects of Military
Subsistence

STUDY NO. 4

Investigation of Selective Media
and Confirmatory Techniques for
Bacillus cereus

PROBLEM:

Bacillus cereus has been an important agent in foodborne illnesses in Europe for many years. In the United States, however, confirmed outbreaks of foodborne illnesses caused by this organism have been extremely rare. Although there are few confirmations of *B. cereus* in foodborne illnesses, the results of several surveys of food items show that *B. cereus* can be isolated from numerous foods and that it grows well under varying conditions. These observations raise the possibility that errors may be occurring in assigning the organism its proper place as a foodborne illness agent. The lack of generalized awareness of the organism's potentiality to cause foodborne illness and of suitable methodology for isolation may partially explain this failure.

RESULTS AND DISCUSSION OF THE RESULTS:

Two recently developed selective media for the isolation and enumeration of *B. cereus* were studied and compared. Basis of comparisons were the ease of medium preparation, stability of medium, general handling characteristics, and efficiency in suppressing growth of organisms other than *B. cereus*. While it is recognized that these are subjective comparisons, it was found that the KG agar of Kim and Goepfert was superior to the MYP agar of Mossel et al. for these factors. Both media performed equally well for the isolation and enumeration of *B. cereus*. A limited survey of the incidence of the organism in ground beef was instituted, and is presently in progress.

In effort to develop diagnostic antisera to the spores of *B. cereus* for use in food microbiology, difficulty was experienced in producing a suspension of spores which was free of vegetative cells; however, this was successfully resolved. An adequate stock of *B. cereus* spore suspension, estimated to be 99.5% pure, is presently on hand.

CONCLUSIONS AND RECOMMENDATIONS:

Evaluations of the two selective media (KG and MYP) indicate that KG medium is highly acceptable for isolation and enumeration of *B. cereus* from food products. It is recommended that the KG medium be used in Army Medical Laboratories in the microbiological examination of foods suspected of having caused foodborne illness.

The foods procured by DoD to test for the presence of *B. cereus* should be extended and expanded to provide a data base on this organism. Efforts should be continued on the development of diagnostic antisera for the definitive identification of presumptive *B. cereus* isolated from food products. The purified spore suspension could be treated and the outer spore coat, inner spore coat, and intraspore components released. The protein profile of the lysed spore suspension could then be examined by polyacrylamide disc gel electrophoresis and isoelectric focusing. Following evaluation of the protein profile patterns under several different sets of conditions, the purification of various protein components by classical techniques of molecular sieve column chromatography, ion exchange column chromatography, and preparative isoelectric focusing techniques could be attempted. Purified spore suspension proteins would then be tested for the production of antibody in rabbits.

PUBLICATIONS:

None

PUBLICATIONS UNDER WORK UNIT 041:

- STUDY NO. 1 Fowler, J.L., J.F. Foster and M.L. Henderson. Microbiology of specification and commercial precooked frozen meals. LAIR Rpt 26, 1975.
- STUDY NO. 2 Fowler, J.L., P.B. Ruckh, T.G. Murnane and W.F. Ganz. Report of analyses of 1972 Microbiological Data Collection Program. USAMRNL Lab Rpt 339, 1973.
- Fowler, J.L., P.B. Ruckh and T.G. Murnane. A Computerized Food Microbiological Data Collection Program and its potential in formulating data for microbiological standards. J. Am. Vet. Med. Assn. 165: 1000, 1974.
- STUDY NO. 3 Fowler, J.L., R.E. Thomas, J.J. Jorgensen and D. Stutzman. Microflora of prepared salads and specialty items procured for use by DoD installations. USAMRNL Lab Rpt 338, 1973.
- Fowler, J.L. and W.S. Clark, Jr. Microbiology of delicatessen salads. J. Milk Food Technol. 38: 146, 1975.
- Fowler, J.L. and J.F. Foster. A microbiological survey of three fresh green salads - Can guidelines be recommended for these foods? J. Milk Food Technol. 39: 111, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISB'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 01 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	61101A	3A161101A91C		00	042		
B. CONTRIBUTING							
C. CONTRIBUTING	None						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Long Term Cryopreservation of Platelets for Immediate Field Use							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine, 012900 Physiology, 008800 Life Support							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 01		78 01		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
EXPIRATION:				76		.8	
B. NUMBER ^a				FISCAL		.3	
C. TYPE:				YEAR		9	
D. AMOUNT:				CURRENT		2.0	
E. KIND OF AWARD:				77		51	
F. CUM. AMT.							
21. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				ADDRESS ^a Blood Research Division			
				Department of Surgery			
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Zuck, Thomas F., LTC, MC			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Platelet Storage; (U) Cryopreservation; (U) Blood Storage; (U) Massive Transfusion; (U) Platelet Transfusion; (U) Traumatic Hemorrhage							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Massive transfusion of stored blood following severe combat injuries leads to dilutional thrombocytopenia and increased bleeding secondary to the lack of platelets. Platelet transfusions can correct this defect but conventional liquid platelet preservation is impractical for field use since platelets are not viable if stored for more than 72 hours. This study explores methods for freezing platelet concentrates so they may be preserved indefinitely and infused without time-consuming washing procedures.</p> <p>24. (U) Studies are in three areas: a) trials of combinations of cryoprotective agents and precise freezing and thawing rates which will satisfactorily preserve platelet viability and function; b) establishment of <u>in vitro</u> and <u>in vivo</u> animal tests which will predict clinical effectiveness in humans; and c) establishment of the conditions for preparing platelet concentrates which will maximize platelet yield and prevent the loss of large platelets which are known to resist injury better than small platelets.</p> <p>25. (U) 76 01 - 76 09 a) Testing of cryopreservation strategies has been delayed pending acquisition of a liquid nitrogen freezer. b) <u>In vitro</u> methods for testing platelet viability have been standardized. Serotonin uptake, osmotic shock resistance, and platelet morphology index discriminate between fresh platelets and those damaged by metabolic poisons or prolonged liquid storage. These tests will be used to screen different cryopreservation methods. c) The conditions for preparing platelet concentrates have been established which optimize platelet yield (87% after centrifugation at 1600 rpm for 8 minutes) and preserve the platelet size distribution and the fraction of large platelets.</p>							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent Research

WORK UNIT NO. 042 Long Term Cryopreservation of Platelets for Immediate Field Use

The following investigations are being conducted under this Work Unit:

STUDY NO.	1	Cryopreservation Strategies
STUDY NO.	2	<u>In Vitro</u> Viability/Function Testing
STUDY NO.	3	Nonhuman <u>In Vitro</u> Viability and Function Testing
STUDY NO.	4	Maximization of Platelet Harvest

Massive transfusion of stored blood following severe combat injuries leads to dilutional thrombocytopenia and increased bleeding secondary to the lack of platelets. Platelet transfusions can correct this defect but conventional liquid storage of platelets is impractical for field use since platelets are only viable when stored for less than 72 hours. Studies under this work unit explore methods for freezing platelet concentrates so that they may be preserved indefinitely and infused without time-consuming washing procedures.

Four methods reported by other laboratories to be of value in assessing viability and function of stored platelets have been established and evaluated in vitro. Serotonin uptake, resistance to osmotic shock, and platelet morphology appear to be the most reproducible and, therefore, of potential value. Chromium uptake of stored platelets proved unpredictable.

In order to maximize harvest of heavier hemostatically active platelets, which are also larger, platelet size distribution studies were performed on blood from normal donors, and on platelet concentrates prepared by graded centrifugation. The separation forces which yield platelet concentrates with optimal size distribution have been determined.

BODY OF REPORT

WORK UNIT NO. 042

Long Term Cryopreservation of Platelets for Immediate Field Use

STUDY NO. 1

Cryopreservation Strategies

PROBLEM:

It is widely accepted that once surgical hemostasis is achieved, thrombocytopenia is the most common cause of generalized bleeding following massive transfusions for severe combat injuries. Platelets (thrombocytes) are labile and remain viable less than 24 hours if stored at 4°C in whole blood or packed red cells. The effective storage interval can be extended to 72 hours by separating platelets from freshly shed blood followed by careful storage in the liquid state at 4° or 20°C. However, for field use outside of the United States, even this period is too fleeting to provide effective support for combat forces. The objective of this work unit is to develop strategies to store platelets in the frozen state using cryoprotective agents in sufficiently low concentrations that they would not have to be removed prior to transfusion. All current methods of frozen platelet storage require a washing procedure immediately prior to infusion. The washing technology is not available with limited resources of field hospitals. All of the studies of this work unit relate to this objective.

RESULTS AND DISCUSSION OF THE RESULTS:

This study has been delayed pending the delivery of a liquid nitrogen controlled rate freezer by the contractors. Current estimate of delivery date is indefinite.

CONCLUSIONS:

None

RECOMMENDATIONS:

As freezing equipment becomes available, information should be applied to other studies of this work unit to screen combinations of cryoprotective agents.

PUBLICATIONS:

None

STUDY NO. 2

In Vitro Viability/Function Testing

PROBLEM:

The objective of this study is to develop an in vitro system for assessing the viability and function of cryopreserved platelets.

RESULTS AND DISCUSSION OF THE RESULTS:

Four different methods for measuring platelet viability have been established and tested with fresh and "damaged" platelets: a) osmotic shock resistance, b) ^{14}C -serotonin uptake, c) $^{51}\text{Chromium}$ uptake, d) platelet morphology index. The range of values for fresh normal platelets were determined. Platelets were damaged by exposure to metabolic poisons (potassium fluoride (KF) and sodium cyanide (NaCN)), and by storage at 4°C , 37°C (4 hours) and at room temperature (72 to 96 hours). Platelets predicted to be nonviable (e.g., 72 hour room temperature storage with final pH less than 6.0) showed essentially no osmotic shock resistance (normal > 50%); essentially no serotonin uptake (normal > 1.5 nanomoles serotonin/minute/ 10^6 platelets), unpredictable chromium uptake (2-3 picograms chromium/minute/ 10^8 platelets; normal 1-3 picograms chromium/minute/ 10^8 platelets); and poor platelet morphology indices (100-200; normal > 350).

CONCLUSIONS:

Osmotic shock resistance, ^{14}C -serotonin uptake and platelet morphology index correlate well with platelet viability and correlate well with each other. The $^{51}\text{Chromium}$ uptake test does not correlate with platelet viability and is therefore discarded as an in vitro screening procedure.

RECOMMENDATIONS:

In vitro platelet viability tests should be used to screen cryopreservation strategies and methods which look promising.

PUBLICATIONS:

None

STUDY NO. 3

Nonhuman In Vivo Viability and Function Testing

PROBLEM:

Conventional tests of platelet viability and function correlate only roughly with clinical effectiveness. Animal models must be developed

to verify the usefulness of cryopreserved platelets. The correction of bleeding in animals (rabbits) rendered thrombocytopenic is potentially useful for this purpose.

RESULTS AND DISCUSSION OF THE RESULTS:

This study is in a preliminary state. An animal model is being developed.

CONCLUSIONS:

None

RECOMMENDATIONS:

If the model proves successful, it should be used as an in vivo assay of the effectiveness of platelet freezing strategies.

PUBLICATIONS:

None

STUDY NO. 4

Maximization of Platelet Harvest

PROBLEM:

Large platelets are heavier and more active metabolically and functionally than small platelets. The preparation of platelet concentrates involves differential centrifugation which may potentially remove the large, heavy platelets. To insure optimal platelet concentrates, specific preparatory methods must avoid the loss of large platelets and maintain the platelet size distribution (PSD). In order to develop baseline data to determine optimal preparation and storage manipulations accurately, the effects of various anticoagulants on platelets from normal donors were investigated.

RESULTS AND DISCUSSION OF THE RESULTS:

The PSD in 43 normal subjects has been characterized (Table 1). In healthy individuals the PSD is lognormal as evidenced by a bell shaped curve on a log scale, a linear log probit plot, and measurements of the coefficients of skewness and Kurtosis which are not different from zero. Anticoagulants may affect the PSD and blood collected in ethylenediaminetetraacetate (EDTA) has an artifactually larger platelet mean cell volume (MCV) (Table 2) than blood collected in balanced citrate, 3.8% sodium citrate, acid-citrate-dextrose (ACD), or citrate-phosphate-dextrose (CPD). Platelet size is stable for at

least 6 hours in balanced citrate or 3.8% citrate, but increases over time in EDTA. Platelet size does not change until blood is centrifuged at forces in excess of 1,000 x g for 10 minutes. Above this force the mean cell volume (MCV) and fraction of large platelets decreases, though the lognormal size distribution is preserved. This implies that even when platelet size decreases it is not the result of a selective loss of large platelets. Centrifugation at 660 x g for 8 minutes to prepare platelet rich plasma resulted in 87% yield with the optimal preservation of platelet size distribution.

TABLE 1
Platelet Size Distribution in Normals

<u>n=43</u>	<u>Average</u>	<u>95% Range</u>	
MCV (geometric)	5.82 fl	4.62	7.01
Std. Dev. (geometric)	1.73 fl	1.63	1.83
CV (geometric)	31.2%	28.3	34.2
MCV (arithmetic)	6.76 fl	5.27	8.25
Std. Dev. (arithmetic)	3.90 fl	2.95	4.86
CV (arithmetic)	57.7%	53.0	62.4
Coeff. skewness	-.012	-.252	.228
Coeff. Kurtosis	-.113	-.368	.141
% Megathrombocytes	8.1%	1.2	15.0

TABLE 2
Effect of Anticoagulant on Platelet Size*

	<u>0 hr</u>	<u>3 hr</u>	<u>6 hr</u>
Balanced citrate	5.81	5.75	5.69
3.8% NaCit	5.83	5.58	5.74
EDTA	6.35	6.81	7.09
"Blue Top Tubes"	6.19	5.71	6.01
ACD	5.77	—	—
CPD	6.05	—	—

*The mean cell volume of platelets collected in various anticoagulants is given for 0 hr storage (immediate) and for samples kept at room temperature for 3 and 6 hours.

CONCLUSIONS:

Platelets prepared by centrifugation by centrifuging whole blood at approximately 660 x g for 8 minutes (first spin), and 3200 x g for 8 minutes (second spin) appears to make optimal concentrates. These

procedures preserve the baseline platelet size distribution and should insure both the optimum yield and function of the platelet concentrate.

RECOMMENDATIONS:

Baseline data developed in this study should be translated into preparation and evaluation techniques for cryopreserved platelets.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISSEM INSTR*	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
76 03 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61101A	3A161101A91C	00	043			
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (precede with Security Classification Code)*							
(U) The Effect of Gastrointestinal Hormones on Gastrointestinal Function							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
002300 Biochemistry, 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 03 01		CONT		DA			
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER:				76		.5	
c. TYPE:				77		.1	
d. AMOUNT:				CURRENT		20	
e. KIND OF AWARD:				77		.5	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Wicks, R. C., MAJ, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME: Herman, R. H., COL, MC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Combat Wounds, (U) Gastrointestinal Function, (U) Gastrointestinal Surgery, (U) Gastrin, (U) G.I. Enzymes, (U) Abdominal Injuries							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23(U) Dietary substances cause changes in enzyme activities in the small intestine. Dietary substances cause the release of numerous hormones from the gastrointestinal (G.I.) tract. The G.I. hormone, gastrin, has a trophic action on the small intestine. In the absence of gastrin the small intestine atrophies and disaccharidases decrease in activity. It is possible that dietary substances affect small intestinal enzymes via the stimulation of gastrin. Healing of combat-incurred G.I. tract injury may be facilitated by the administration of gastrin.</p> <p>24(U) The effect of gastrin on small intestinal enzymes of the partially gastrectomized dog will be studied. Appropriate analysis will be done to ensure that the animal is gastrin deficient. Various chemical forms of gastrin will be used to determine if small intestinal enzyme activity is increased. The response to dietary substances with and without gastrin will be tested. If gastrin is effective other hormones of the G.I. tract will be tested.</p> <p>25(U) 76 03 - 76 10 Studies to date demonstrate the feasibility of maintaining a partially gastrectomized dog in sufficient good health so that small intestinal tissue can be obtained for analysis on a periodic basis. If gastric resection is too drastic the small size of the resulting stomach makes food intake insufficient to maintain weight. If an insufficient amount of stomach is removed the animal will not become gastrin deficient. This surgical problem is a limiting factor and necessitates careful judgment when performing subtotal gastrectomy.</p>							

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	043	The Effects of Gastrointestinal Hormones on Gastrointestinal Function

The following investigations have been conducted under this work unit:

STUDY NO. 1 In Vivo Study of the Role of Gastrin in the Control of Small Intestinal Mucosal Enzymes on the Dog.

Gastrin, a polypeptide hormone, synthesized in the gastric antrum, has recognized trophic effects in the small intestine in rats. Gastrin deficiency due to absence of enteral food or antral tissue leads to gastrointestinal atrophy. If the trophic effect of gastrin is required for normal intestinal protein synthesis, then gastrin deficiency (resulting from gastric or intestinal resection) will lead to abnormal protein and enzyme synthesis and subsequent abnormal gastrointestinal function. The acutely injured soldier with abdominal injuries requiring gastric or intestinal resection may be gastrin deficient and consequently postoperative gastrointestinal function may be favorably influenced by gastrin therapy. We will study the effects of gastrin on intestinal glycolytic enzymes in the dog made gastrin-deficient by antrectomy. Pilot studies are in progress to master the surgical techniques necessary to perform serial small intestinal biopsies.

BODY OF REPORT

WORK UNIT NO. 043

The Effects of Gastrointestinal
Hormones on Gastrointestinal Function

STUDY NO. 1.

In Vivo Study of the Role of Gastrin
in the Control of Small Intestinal
Mucosal Enzymes on the Dog.

PROBLEM:

An important aspect of acute gastrointestinal disease involves combat-related abdominal injury and its sequelae. Abdominal injuries are frequent and serious complications of any military operation. In World War II, in one field hospital, wounds of the stomach comprised 416 of 3,154 cases of abdominal injury. The fatality rate was 40%. Approximately 30% of the abdominal injuries consisted of wounds of the small intestine. Approximately 20% of the total number of injuries required partial resection of the gastrointestinal tract. Many patients with abdominal injuries will have altered gastrointestinal function secondary to resection of portions of the intestinal tract. With improved techniques of first aid, evacuation, blood replacement, surgery, and prophylaxis and treatment of infection, we can expect an increased number of combat-wounded soldiers to reach the postoperative period. At this point only general supportive measures are available and no specific therapy is known which can hasten healing and restore function of the gastrointestinal tract. Food intake, intestinal hormones and intestinal adaptation all make considerable contributions to the recovery process after intestinal resection. Several observations suggest that the antral hormone, gastrin, has trophic effects on the gastrointestinal tract. In rats, gastrin has increased ^{14}C -leucine incorporation into protein, ^{14}C -orotic acid incorporation into RNA, and ^{14}C -thymidine incorporation into DNA. Gastrin trophic effects have been demonstrated in in vitro tissue cultures of rat gastric and duodenal mucosa. Pentagastrin (PG) stimulated epithelial cell growth, decreased cell doubling time, and decreased cell contact inhibition.

Two different laboratories have demonstrated the importance of food intake in regulating small intestinal enzymes. In rat, intravenous hyperalimentation decreased intestinal maltase and sucrase activities. Tissue gastrin fell concomitantly. The disaccharidases were restored to control levels by PG which suggests that gastrin may control intestinal disaccharidases. Both tissue gastrin and intestinal disaccharidases returned to normal after oral feeding. Previous studies in this laboratory have demonstrated increased activity of jejunal glycolytic enzymes in response to carbohydrate meals. Specific sugars caused adaptive changes in the enzyme most concerned with the metabolism of the specific substrate and was in addition to a

generalized increase in enzyme activity attributed to calories alone. Since food intake influences gastrin and intestinal enzymes, and since gastrin has documented trophic effects in the gut, it is conceivable that gastrin has a generalized effect on protein synthesis in the gut. If the trophic effect of gastrin is required for normal intestinal protein synthesis, then gastrin deficiency states occurring as a consequence of gastric or intestinal resection, could result in abnormal protein synthesis and subsequent maladaptation of intestinal enzymes. There is ample in vivo and in vitro support for a gastrin trophic effect. There is also evidence to suggest that food intake is important in determining the level of intestinal enzymes and the amount of tissue gastrin. The acutely injured soldier who has lost variable amounts of stomach and small intestine has reduced intestinal function by virtue of the surgical resection. The enzyme activities in the remaining gut are responsive to food intake and gastrin, both of which have been reduced by the surgical procedure. It is reasonable to believe that replacement of gastrin will restore intestinal enzymes to normal and hasten restoration of gastrointestinal function. We will study the role of gastrin in the control of selected intestinal enzyme activities. Dogs will be made gastrin deficient by antrectomy. We will determine if any alteration of the intestinal enzyme activity can be reversed by administration of exogenous hormones (gastrin G-17 and PG) or by endogenous small intestinal gastrin (ordinarily a minor source of the hormone) released by duodenal feeding of specific nutrients.

RESULTS AND DISCUSSION OF THE RESULTS:

To date two animals have been studied with regard to the technical surgical problems necessary in this project. We can successfully obtain adequate tissue with our biopsy procedure. Although the animals have tolerated antrectomy well certain problems have occurred such as fistula formation between the skin and duodenum related to canula pressure necrosis. The surgical problems appear to have been satisfactorily solved and our study is in progress.

CONCLUSIONS:

This is a new project which is in its early stages so no conclusions are available.

RECOMMENDATIONS:

The study should be pursued since this is a unique and critical military problem.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9. LEVEL OF SUM A. WORK UNIT	
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61101A	3A161101A91C	00	045			
b. CONTRIBUTING							
c. CONTRIBUTING	NONE						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Development of a Model Host System of American Cutaneous Leishmaniasis							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002600 Biology; 010100 Microbiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 08		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER ^a Not Applicable				76		2.2	
c. TYPE:				77		.5	
d. KIND OF AWARD:				77		3.8	
e. CUM. AMT.						100	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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ADDRESS ^a Presidio of San Francisco, CA 94129				Department of Tropical Medicine			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME ^a Wilson, H.R.			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3560			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Mellick, Paul W., MAJ			
				NAME: Moussa, M.A., LTC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Animal Model; (U) <u>Leishmania braziliensis</u> ;							
(U) <u>Leishmania mexicana</u> ; (U) Immunosuppression; (U) Pathology; (U) Infectivity							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To develop a model host system of American cutaneous leishmaniasis for better understanding of the pathogenesis, immunity and transmission of the disease. Knowledge to be gained from these studies is essential to the development of improved measures for the protection and treatment of military personnel serving in endemic areas overseas.</p> <p>24. (U) Efforts will be made to standardize and optimize infection of <u>Leishmania mexicana</u> and <u>L. braziliensis</u> in hamsters or other suitable hosts. Time of appearance, development, and metastasis of lesions will be monitored. Gross and histopathologic aspects of the infection will be ascertained. Effects of selected immunosuppressants in enhancing the infection will be studied.</p> <p>25. (U) 75 07 - 76 09 Amastigote and promastigote-induced infections of <u>Leishmania braziliensis</u> and 2 strains of <u>L. mexicana</u> have been quantitated in the hamster, and conditions essential to achieving reproducible infections with rapid onset were defined. Amastigotes were 10-100X more infective than promastigotes obtained from primary culture and consistently yielded more reproducible infections. Intradermal inoculation was superior to subcutaneous inoculation. Histopathological criteria for differentiation of <u>L. braziliensis</u> and <u>L. mexicana</u> lesions were established. The African White Tailed Rat, <u>Myiostomys albicaudatus</u>, presented no advantages over the hamster as a potential model host of <u>L. braziliensis</u> infection.</p>							

ABSTRACT

PROJECT NO. 3A161101A91C

In-House Independent Laboratory
Research

WORK UNIT NO. 045

Development of a Model Host
System of American Cutaneous
Leishmaniasis

Factors affecting onset, growth and reproducibility of cutaneous lesions of Leishmania braziliensis and L. mexicana in hamsters were defined and quantitated. The intradermal route of inoculation was superior to the subcutaneous route in inducing infection. Amastigotes obtained from trypsinized lesions were 10 to 100 times more infective than promastigotes from primary culture. Prolonged serial cultivation substantially reduced promastigote infectivity. Lesions appeared within 6 days following intradermal injection of 10^6 amastigotes or within 14 days following 10^6 promastigotes from primary culture. Amastigote-induced infections were more reproducible than promastigote-induced infections. Infections of the paw provided better growth and were more easily quantitated than infections of the nose, ear, or flank. A new staining procedure, Modified Whipf's Polychrome, was developed to demonstrate optimally both parasite and tissue components of lesions. Certain histopathological differences between L. braziliensis and L. mexicana infections in the hamsters were delineated. Evaluation of the African White-Tailed Rat, Myiostomys albicaudatus as an alternate model host for L. braziliensis revealed no advantage over the hamster. Development of the model host system will expedite our discerning methods of prevention and treatment of cutaneous leishmaniasis encountered by American service personnel in Central and South America.

BODY OF REPORT

WORK UNIT NO. 045

Development of a Model Host
System of American Cutaneous
Leishmaniasis

PROBLEM:

American cutaneous leishmaniasis is a sand fly-borne parasitic disease periodically encountered by U.S. service personnel stationed in endemic areas in Central and South America. There are neither satisfactory chemotherapeutic nor adequate preventive measures to insure the protection of susceptible personnel. The development of improved methods for prevention and treatment of this disease, however, is contingent upon better understanding of the mechanisms involved in its pathogenesis, immunity, and transmission. The lack of progress of these developmental efforts is attributed to the absence of a suitable model host system of this disease. Although studies have been conducted on experimental infections and pathology of Leishmania braziliensis and L. mexicana in animals, no successful attempts have been made to develop such a model system. This study is directed to the development of a model host system of this disease with strains of Leishmania representative of endemic areas.

RESULTS AND DISCUSSION OF THE RESULTS:

Cutaneous infections of a Brazilian strain of L. braziliensis originating from a human mucocutaneous lesion and 2 strains of L. mexicana, one of Costa Rican and the other of Brazilian origin were produced by multiple cutaneous inoculations of individual hamsters with quantitated inocula. Factors influencing infectivity of these 3 strains to hamsters were delineated. The forepaw was superior to the nose, ear and flank as the site of inoculation because of ease and accuracy of measurement of lesions and more rapid growth. The intradermal inoculation route, which parallels inoculation of the parasite by the sand fly vector, proved superior to the subcutaneous route in establishing infections. Amastigotes were 10 to 100 times more infective than promastigotes from primary cultures. Inocula of 10^6 parasites from these sources produced lesions within 6 days and 11 to 27 days, respectively. Onset times of lesions of L. braziliensis were more than doubled after 14 passages in culture, indicating decreased infectivity.

Infectivity of the 3 Leishmania strains in the hamster were compared. Following inoculation with 10^6 amastigotes, growth of lesions of both L. mexicana strains was rapid and sustained during the 6 week duration of the study. Similarly, rapid growth of the L. braziliensis lesions was achieved from comparable inocula during the first 14 to 18 days, but slowed thereafter leveling off by 4 to 6 weeks.

Reproducibility of amastigote and promastigote-induced L. braziliensis infections in the hamster was also confirmed. Dose levels of 10^4 amastigotes and 10^6 promastigotes resulted in lesions with median onset times of 8.4 and 11.0 days which compared closely with those previously observed. Growth patterns were also similar. However, the growth of promastigote-induced infections were more variable than amastigote infections ($p < .01$).

Some hamsters infected with 10^4 L. braziliensis-amastigotes demonstrated moderate regression of lesions. Both immediate and delayed-type hypersensitivity responses were demonstrated in these hamsters suggesting an immune response. Such findings have not been previously reported.

Histopathological criteria for differentiation of L. braziliensis and L. mexicana lesions in the hamster were established, utilizing a staining procedure developed for this purpose, Modified Whipf's Polychrome. This stain affords ready recognition of parasites and superior differentiation of connective tissue and other lesion components. Typical, active L. mexicana lesions consisted largely of vacuolated macrophages containing numerous parasites. Varying degrees of infiltration by lymphocytes and plasma cells, not observed in L. braziliensis infections, were also regularly observed. In contrast, only focal collections of vacuolated parasitized macrophages were observed in L. braziliensis lesions; non-vacuolated macrophages made up the bulk of the lesion. Schaumann bodies were frequently noted but were not found in L. mexicana lesions.

The African White-Tailed Rat, Mystromys albicaudatus, was also evaluated as a potential model for L. braziliensis infection. The growth of lesions in M. albicaudatus was variable and required 5 to 8 weeks to appear in contrast to 1 week in the hamsters. These findings, in addition to the difficulty in handling, indicated no distinct advantages over the hamster.

CONCLUSIONS:

Quantitative techniques have been developed, evaluated and proven in the laboratory which permit the manipulation of cutaneous L. braziliensis and L. mexicana infections in the hamster for the first time. Infections of these two parasites were highly reproducible on the basis of onset times and growth of lesions. This completes an important step in the development of the desired model. Slow and variable growth of lesions as well as difficulties encountered in the handling of M. albicaudatus do not favor this species as a potential model host. The finding of moderate lesion regression and hypersensitive responses in L. braziliensis infection of the hamster points to the need to continue development of this animal as a model host of this disease.

RECOMMENDATIONS:

Investigations of leishmanial infection in the hamster should be continued with emphasis on the use of L. braziliensis due to its medical importance and wide range of distribution. Further studies should be done on modifying the course of infection through the use of selected immuno-enhancing or immuno-suppressive agents. The potential utilization of immuno-suppressive agents in intensifying infection and in maximizing the yield of amastigotes from donor animals should be explored. Also, the suitability of the infected hamster for use in transmission studies utilizing a natural vector should be determined.

PUBLICATIONS:

Wilson, H.R., and D.G. Fairchild. Modified Whipf's Polychrome: A Connective Tissue Stain with Special Application for Demonstrating Leishmania. (Submitted for publication.)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DAOE 6072	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUM'Y	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTN ^a	9b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61101A		3A161101A91C		00	
B. CONTRIBUTING						051	
C. WORK UNIT		None					
11. TITLE (Precede with Security Classification Code) ^a							
(U) Influence of Shock on Excretory Function of the Liver							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
012900 Physiology, 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 08		77 06		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				B. PRESENT		C. FUTURE	
B. NUMBER* Not Applicable				76		1	
C. TYPE:				77		0.3	
D. KIND OF AWARD:				77		1	
E. AMOUNT:				77		34	
F. CUM. AMT.				77		34	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Hypovolemic Shock; (U) Bile Salt Secretion;							
(U) Bile Salt Synthesis; (U) Liver Excretory Function; (U) Bile Secretory Rates							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Deterioration of the excretory function of the liver not uncommonly complicates the recovery of the wounded soldier, and frank liver failure may result. Bile salt secretion is one of the most sensitive parameters of the excretory function of the liver. The effects of hypovolemic shock on hepatic synthesis and secretion of bile salt will be investigated, and resuscitative agents and measures to modify adverse effects will be tested.</p> <p>24. (U) A simplified model, to allow long term measurement of hepatic bile salt synthesis and secretion in baboons with intact gallbladders, will be used. These parameters will be assessed during normovolemic control periods and during and after hypovolemic shock. A stream-splitter pump, returning 95% of collected bile in a chronic biliary fistula, will allow continued sampling of bile without interruption of the enterohepatic circulation.</p> <p>25. (U) 75 07 - 76 09 Base-line values for bile salt synthesis and secretion, bile salt pool size and contribution of the gallbladder to bile salt pool size have been obtained in four normovolemic, fasting baboons with chronic biliary fistulas. The liver synthesizes 10 μmoles and secretes 570 μmoles of bile salt per hour. Approximately 40% of hepatic bile enters the intact gallbladder, and the gallbladder contains 1,720 μmoles of the total bile salt pool of 2,100 μmoles. The effects of hypovolemic shock on these parameters will be studied in these animals.</p>							

ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent Research
WORK UNIT NO. 051 Influence of Shock on Excretory Function
of the Liver

The following investigations have been conducted under this Work Unit:

A subhuman primate model (baboon) has been developed which allows quantitation of hepatic excretory function under physiologic conditions. The enterohepatic circulation is maintained, and the gallbladder functions normally. Animals tolerate the procedure well on a chronic basis, and a variety of parameters of liver function have been studied.

Since bile salt secretion is one of the most sensitive indicators of the excretory function of the liver, it has been chosen as a primary variable for investigation. An extensive series of base-line measurements has been completed in preparation for studying the effect of shock on liver function. In chronically maintained, fasted animals, approximately 40% of hepatic bile enters the gallbladder, and the remainder passes directly into the duodenum. The liver secretes 570 μ moles of bile salt per hour, of which 240 enter the gallbladder. The total bile salt pool is 2,100 μ moles, of which 1,720 are sequestered in the gallbladder. In response to a meal, the gallbladder evacuates 83% of its contents over a 110-minute period, and hepatic secretion of bile salt increases to 1,250 μ moles/hr. These data provide the necessary base-line measurements for comparisons in studies on shock.

BODY OF REPORT

WORK UNIT NO. 051

Influence of Shock on Excretory Function of the Liver

PROBLEM:

Severe injury and hypovolemic shock resulting from combat trauma are associated with a significant incidence of multiple organ failure. Massive metabolic insults mediated through the effects of shock and trauma, as well as multiple transfusions, stress the excretory function of the liver. Deterioration of liver function can complicate the patient's clinical response; frank liver failure may result.

Our long-range goals are to investigate the effects of blood loss, trauma, and multiple transfusions on the excretory function of the liver and, ultimately, to determine means of improving the liver's response to shock and stress. Since bile salt secretion is one of the most sensitive indicators of the excretory function of the liver, it has been chosen as the major primary variable for investigation. Study of bile salt metabolism is complicated by the enterohepatic circulation, which, among other things, is affected by the presence of a functioning gallbladder. It was necessary to perfect a primate model which permits the necessary measurements of bile salt metabolism to be made without interrupting the enterohepatic circulation or interfering with normal gallbladder function. This has been accomplished in a baboon model under this Work Unit.

The goal of this study was twofold: (1) to conduct a series of baseline measurements using the model; the measurements can then be used as a standard of reference during experimental periods; and (2) to use the model to determine what effect shock and multiple transfusions have on liver function.

RESULTS AND DISCUSSION OF THE RESULTS:

Four male baboons were prepared, each with a duodenal fistula. Bile from the fistula was passed through an electronic stream-splitter. Five percent of the total bile flow was continuously sampled by the stream-splitter, and the remaining 95% returned to the animal through a tube duodenostomy. In this way, bile flow could be measured and samples collected without interfering with the normal enterohepatic circulation or normal gallbladder function. To assess bile salt secretion rates and bile salt pool size, bile salt labeled with ^{14}C was given to the animals. Bile salt synthesis was measured directly by the "wash-out" technique of Small. Initial technical problems with the model have been overcome, essentially, and the procedure appears to be well tolerated by the animals.

To define bile salt metabolism, each animal was studied immediately following a fatty meal, and, again, after a 12-hour fast. Four parameters were measured: (a) percent of hepatic bile entering the gallbladder, (b) rate of bile salt secretion from the liver, (c) size of bile salt pool and the amount of this pool sequestered in the gallbladder, and (d) percent of gallbladder contents evacuated in response to a fatty meal. Results are expressed as the mean of eight experiments (± 1 SEM), two in each of four animals. Immediately following a fatty meal, there was a total bile salt pool of $2,100 \pm 280$ μ moles contained in the enterohepatic circulation. The liver secreted $1,250 \pm 67$ μ moles of bile salt per hour. None of this bile salt entered the gallbladder. After a 12-hour fast, $1,720 \pm 208$ μ moles of the bile salt pool had been sequestered in the gallbladder, and only 390 ± 28 μ moles remained in the enterohepatic circulation. Bile salt secretion by the liver had fallen to 570 ± 93 μ moles/hr. Of this hepatic bile salt secretion, 240 ± 86 μ moles/hr, or 43%, entered the gallbladder, and the remainder passed into the duodenum. In response to a fatty meal, the gallbladder evacuated $83 \pm 17\%$ of its contents over a 110-minute period, but no bile salt left the gallbladder during the 12-hour fast.

In two animals, the effects of gram-negative sepsis were studied. An 80% reduction in bile salt secretion and a 60% reduction in bile flow were noted 48-72 hours before any clinical evidence made it apparent that the animals were ill. This reinforces the postulate that bile salt secretion is a highly sensitive indicator of liver function.

Preliminary experiments, during which one animal was subjected to hypovolemia by graded phlebotomy suggested that hepatic excretory function is sharply altered during acute moderate hypovolemia associated with hypotension.

CONCLUSIONS:

The nonhuman primate model allows comprehensive study of hepatic bile salt metabolism. The procedure is well tolerated by the animals and does not significantly alter normal physiology. The extensive series of base-line measurements which has been accumulated provides a basis with which to compare effects of shock on liver function during future investigations.

RECOMMENDATIONS:

Study of the effects of hypovolemic shock on hepatic excretory function should be completed.

PUBLICATIONS:

1. Gardiner, B. N. and C. Conaway: Quantitation of gallbladder function and effect on bile salt metabolism in primates. Surg Forum 27:373-375, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6086	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	II. TERMINATION	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61101A	3A161101A91C		00		055	
b. CONTINUATION							
c. CONTINUATION	NONE						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Study of Factors Affecting Calcium-Binding Protein							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry; 002600 Biology; 012900 Physiology; 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 01		76 09 30		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				b. PRECEDING		c. FUNDS (In thousands)	
EXPIRATION:				76		2.0	
b. NUMBER ^a				FISCAL		7T	
c. TYPE				YEAR		CURRENT	
Not Applicable				77		-0-	
d. AMOUNT:				77		-0-	
e. KIND OF AWARD:				f. CUM. AMT.			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Empson, R.N., Jr., CPT, VC			
				NAME: Morrissey, R.L., CPT, VC POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Calcium; (U) Calcium-binding Protein; (U) Vitamin D; (U) Bone; (U) Intestine; (U) Kidney; (U) Pancreas; (U) Immunohistochemistry							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Regulation of calcium (Ca) metabolism involves interaction of several nutrients, organs and hormones; it is not well understood. Comprehension of these dynamics is imperative to prevent decalcification or improve calcification in the many military clinical situations in which Ca metabolism is important. One regulatory factor in Ca metabolism, calcium-binding protein (CaBP), can be visualized in tissue preparations. This study will examine the role of CaBP in normal and pathologic conditions. Results should contribute to understanding Ca metabolism and to treatment of military patients, e.g., with skeletal trauma.</p> <p>24. (U) CaBP will be localized by enzyme immunohistochemistry in a variety of human and animal tissues, and its presence correlated with various physiologic and spontaneous or induced pathologic states. The latter will include Vitamin D deficiency, induced hypo and hypercalcemia, parathyroid supplementation or removal, dietary alterations, kidney and pancreatic disease.</p> <p>25. (U) 75 07-76 09. CaBP specifically induced in the intestinal mucosa by Vitamin D treatment of rachitic chicks was identified and localized. The protein first appeared in nuclei of crypt cells, then in cytoplasm as the absorptive cells matured. Renal CaBP was not depleted by vitamin D deficiency in chicks and may not be completely vitamin D-dependent. Antiserum to chick intestinal CaBP was unsatisfactory in localizing CaBP in biopsies of intestine from humans. Lack of specific antibody to human intestinal CaBP, and loss of personnel experienced with this immunohistochemical technique have led to termination of this work unit. (See Am. J. Path. 82:55a, 1976 and Federation Proceedings 35:746, 1976.)</p>							

ABSTRACT

PROJECT NO. 3A161101A91C

In-House Laboratory Independent Research

WORK UNIT NO. 055

Study of Factors Affecting Calcium-Binding Protein

The objective of this work was to continue to evaluate the distribution of calcium-binding protein (CaBP) in tissues of animals and human patients as an index of calcium metabolism and vitamin D response. The CaBP was localized immunohistochemically with a peroxidase-labeled antibody. By using antibody to CaBP from chick intestine, synthesis of CaBP in the small intestine was shown to be specifically induced by 1-alpha hydroxy cholecalciferol or 1,25 dihydroxy cholecalciferol. The protein first appeared in nuclei of crypt cells and then in cytoplasm as the absorptive cells matured. Total depletion of CaBP from renal distal tubule epithelium did not occur in rachitic chicks, and so new synthesis of CaBP upon repletion with vitamin D could not be demonstrated.

CaBP was not localized consistently in tissue obtained at biopsy from small intestinal mucosa of human patients having altered calcium metabolism. This difficulty can not be surmounted until antiserum specific for human intestinal CaBP is available.

A completely satisfactory technique for electron microscopic localization of CaBP could not be developed during the reporting period.

This work unit is being terminated because of lack of personnel technically qualified in immunocytochemistry.

BODY OF REPORT

WORK UNIT NO. 055

Study of Factors Affecting
Calcium-Binding Protein

PROBLEM:

Immunohistochemical localization of calcium-binding protein (CaBP) with peroxidase-labeled antibody to CaBP provides a useful index of some aspects of calcium metabolism and vitamin D function. Additional information about calcium and vitamin D is needed to improve management of military patients having disease or trauma which affects bone, intestine, or kidney. An understanding of the role of CaBP may provide one measure which will increase our understanding of the entire system.

In our efforts to use CaBP localization in seeking the desired information, we have recognized that the anti-CaBP antiserum in use was produced in rabbits, with CaBP from human kidney as antigen. This antiserum localized CaBP in kidneys from man and from each of several other animal species. The antibody has limitations, however, in localization of CaBP in intestine; it is erratic in human intestine and does not work at all in intestine of other species. Specific objectives of this work unit were: 1) to test a new rabbit antiserum against chick intestinal CaBP, in the hope that it would enable us to localize intestinal CaBP in man and laboratory animals; 2) to determine with this antiserum whether renal CaBP was vitamin D responsive; 3) to supplement other LAIR studies (WU 059, Project Area 71P-01 and WU 060, Project Area 91C) of calcium and vitamin D metabolism with CaBP localization data; 4) to obtain improved procedures for electron microscopic localization of CaBP.

RESULTS AND DISCUSSION OF THE RESULTS:

The new antiserum against chick intestinal CaBP was highly successful in labeling CaBP in the small intestine of chick, rat, and mouse. It discriminated cleanly between vitamin D-deficient chicks (no intestinal CaBP) and normal or repleted, formerly deficient chicks. In the absorptive cells, CaBP was present throughout the cytoplasm and occasionally in the nucleus. Goblet cells and mucus did not contain CaBP. The antiserum yielded only negative results in attempts to localize CaBP in human intestinal biopsies, however. Use of this antiserum in vitamin D-deficient chicks which were subsequently repleted with either 1-alpha hydroxy cholecalciferol or 1,25 dihydroxy cholecalciferol revealed that CaBP synthesis was restricted to, and correlated temporally with, maturation of intestinal absorptive cells. CaBP-containing nuclei were numerous in immature crypt cells, which suggests a regulatory role operative between vitamin D stimulus and CaBP synthesis in the cytoplasm.

In the kidney, even vitamin D-deficient animals had localizable CaBP. Since the technique was not quantitative, it was not possible to determine vitamin D dependency of renal CaBP. However, previous work with

vitamin D toxicity suggests some relation between CaBP and the pathologic calcification of renal tubule cells.

Electron microscopy of kidneys from vitamin D-toxic animals showed early calcium deposits to be associated with mitochondria. Attempts to localize CaBP with electron microscopy were not completely satisfactory. The best results were achieved after fixation with 2% paraformaldehyde; ultrastructural preservation was only fair.

A combined technique was developed for simultaneous demonstration of both CaBP and calcium deposits in the same tissue section with light microscopy.

Surveys for CaBP in tissues of rhesus monkeys, swine, chicks, and rats are in progress with the new antiserum to chick intestinal CaBP.

Renewed attempts to demonstrate CaBP in fetal (unmineralized) bone were unsuccessful but revealed the first presence of CaBP in fetal rat kidney on the 18th day of gestation.

CONCLUSIONS:

This work unit has revealed that in the vitamin D-intestinal CaBP interaction, the steroid hormone (vitamin D) induces synthesis of a specific protein (CaBP). The work also demonstrated that the induced protein is present in the nucleus before it appears in the cytoplasm. This unusual observation requires confirmation and further exploration.

It is apparent that antibody to species-specific CaBP is necessary for reliable localization of intestinal CaBP, unlike the situation in the kidney.

The loss of a coinvestigator and two trained technicians during the past FY has indicated that this work cannot be accomplished without personnel highly specialized in immunochemistry techniques.

RECOMMENDATIONS:

Because of lack of technical help, it is recommended that this line of investigation be terminated and the results to date be published. Efforts should be renewed under related work units to obtain sufficient CaBP from human intestine to produce specific antisera. These antisera would have a high probability of permitting improved clinical evaluation of the intestinal CaBP system in human patients with abnormal calcium or vitamin D metabolism.

PUBLICATIONS:

1. Morrissey, R.L., R.N. Empson, Jr., T.J. Bucci and D.D. Bikle: Immuno-enzymatic localization of calcium binding-protein (CaBP) in duodenal epithelium of rachitic and vitamin D-repleted chicks. Federation Proc. 35:746, March 1976.
2. Empson, R.N., T.J. Bucci, R.L. Morrissey and J.S. Chandler: Restriction of renal lesions induced by vitamin D₃ to tubules containing calcium binding-protein. Am. J. Path. 82:55a, February 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9. LEVEL OF SUM A. WORK UNIT	
75 07 01	D. Change	U	U	DA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO 9B. SPECIFIC DATA - CONTRACTOR ACCESS	
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
6. PRIMARY	61101A	3A161101A91C	00	060			
XXXXXX							
XXXXXX	None						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Vitamin D, Calcium and Phosphorus Metabolism (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry; 002600 Biology; 0016800 Toxicology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 04		CONT		DA		C In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (in thousands)	
D. NUMBER: Not Applicable				76		2.0	
C. TYPE:				FISCAL		77	
E. AMOUNT:				CURRENT		0.4	
F. CUM. AMT.				77		1.8	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Zolock, D.T., CPT, MS			
				NAME: POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ^a							
(U) Combat Bone Injuries; (U) Vitamin D; (U) Calcium; (U) Phosphorus; (U) Mineral Metabolism							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Metabolic effects of vitamin D₃ and its metabolites include induction of calcium binding protein (CaBP) in intestine and kidney and increased intestinal calcium absorption. Bone and joint injuries account for approximately 25% of the operations performed at combat surgical hospitals. At current hospitalization costs (\$138.15/day) and a conservative estimate of \$20.00/day for soldier compensation, the bone and joint injuries treated in the Army between 1964 and 1969 would cost \$768,877,354.70. Also, in FY 75, Army dental facilities performed 859,722 tooth restorations at a cost of \$6,448,290.00. It is anticipated that nutritional and medical therapeutic improvements resulting from this research may improve these statistics, through improved absorption and retention of calcium and phosphorus.</p> <p>24. (U) The influence of nutritional factors (i.e. dietary calcium, phosphorus and vitamin D) on intestinal CaBP concentration and calcium binding activity will be studied in man and animal models. Also, in vitro and in vivo animal studies will be performed to better define the mechanism of calcium transport and homeostatic regulation.</p> <p>25. (U) 75 07 - 76 09 The most significant findings in the current FY are the following: 1) CaBP synthesis occurs in intestinal absorptive cells rather than goblet cells. 2) 1,25-Dihydroxy vitamin D₃ causes a significant increase in the ability of the intestine to transport calcium prior to the initiation of detectable levels of CaBP. 3) Cyclohexamide will prevent induction of CaBP by 1,25-dihydroxy vitamin D₃ but fails to prevent increased intestinal transport of calcium. 4) Chick pancreas CaBP is antigenically identical and similar in chemical and physical properties to chick intestine CaBP. 5) Cross antigenicity has been demonstrated between tissues of different animal species, thus enhancing the probability that a suitable antibody can be developed for assay of CaBP in human intestinal biopsy specimens.</p>							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Research
WORK UNIT NO.	060	Vitamin D, Calcium and Phosphorus Metabolism

The following studies have been conducted under this work unit in the current fiscal year.

STUDY NO. 7	Chemical and Physical Properties of CaBP from Human Renal Tissue
STUDY NO. 9	Kinetics and Localization of CaBP Appearance in Chick Intestine Following 1, 25-Dihydroxycholecalciferol Administration

Study No. 7. A new simplified purification procedure has been used to prepare pure calcium binding proteins (CaBP) from intestinal preparations from chick, monkey, and pig. Rabbit antiserum has been prepared against the chick intestinal CaBP and the antisera for monkey and pig intestinal CaBP are in preparation. Cross reactivity studies using human kidney anti-CaBP serum and chick intestinal anti-CaBP serum have demonstrated antigenic similarities between CaBP from various animal species. A semi-quantitative method for measuring CaBP concentrations has been developed using double immunodiffusion Ouchterlony plates. The sensitivity of detection is 50 to 100 times more sensitive than Chelex-⁴⁵Ca competitive binding assay. A CaBP has been demonstrated in the chick pancreas which is not only antigenically identical to that found in the chick intestine but also similar in its chemical (pI approximately 4.5) and physical (mol. wt. approximately 30,000) properties.

Study No. 9. Experiments have been performed which demonstrate that CaBP is synthesized in intestinal epithelial cells, where it frequently occurs in the nucleus during the initial induction phase in the rachitic animal. Intestinal cell responsiveness is apparently related to the "age" of the particular cell. Following a dose of 1,25-dihydroxy vitamin D₃, calcium transport is initiated at least two hours before CaBP can be shown by a method capable of detecting as little as 64 ng of CaBP. Also, cyclohexamide will prevent the induction of CaBP by 1,25-dihydroxy vitamin D₃ even 12 hours after treatment. The intestinal transport of calcium is not blocked under the same experimental conditions and in the same experimental animals. Thus, it appears that at least part of the metabolic effects of 1,25-dihydroxy vitamin D are independent of CaBP.

BODY OF REPORT

WORK UNIT NO. 060

Vitamin D, Calcium and Phosphorus
Metabolism

STUDY NO. 7

Chemical and Physical Properties
of CaBP from Human Renal Tissue

PROBLEM:

Our understandings of calcium, phosphorus, and vitamin D and their interrelationships in metabolism and basic body functions are inadequate. These inadequacies hinder the military medical prevention and therapy of soldiers who have disorders, such as bone and joint injuries (especially stress fractures), dental disease, osteoporosis (primarily due to immobilization and post-traumatization), and general malnutrition. Scientific advancement in our understandings of calcium, phosphorus, and vitamin D metabolism will lead to the possibility of either preventing these disorders or shortening the time of recovery. Therefore, the military will have a substantial economic advantage and more manhours to accomplish primary missions.

Calcium ions are necessary for (a) the preservation of the skeletal structure (bone utilizes 99% of the total body calcium), (b) normal muscle contraction (muscle utilizes 0.3% of the total body calcium), (c) normal transmission of nerve impulses, (d) normal cell replication and growth control, and (e) blood coagulation. Calcium ions also decrease both neuromuscular excitability and capillary and cell membrane permeability, as well as activate various enzymes which influence the release of certain peptide hormones and other regulatory substances. In order for these functions to be maintained, calcium homeostasis must be established in the body. The factors of greatest importance to calcium homeostasis are those concerned with calcium absorption, deposition in bone, mobilization from the bone, and excretion. Vitamin D (especially its active metabolite 1,25-dihydroxycholecalciferol), certain hormones, and phosphorus play an important role in regulation of calcium metabolism. Calcium binding proteins (CaBP) appear to be vitamin D dependent and have been implicated in calcium absorption. The proteins are also present in kidney, which suggest their possible role in renal retention of calcium.

It is anticipated that results of this research study will define more precisely the functional properties of CaBP and the mechanism of calcium binding. Thereby, they will establish a better understanding of the overall mechanism of calcium absorption and retention. This understanding is essential to the design of preventive and therapeutic measures for related medical problems. The results will also be extremely helpful in determining whether or not human renal CaBP is similar to other CaBP including the vitamin D dependent CaBP. If so,

this will simplify tremendously the approach to studies of human CaBP in calcium absorption and excretion because some experiments which are impossible to perform on humans may be carried out on other animal species.

RESULTS AND DISCUSSION OF RESULTS:

Fresh autopsy and surgical specimens of human kidney necessary to isolate pure human renal CaBP in sufficient quantities for investigation have been limited. Therefore, the majority of the following studies were conducted on CaBP from other animal species. Chick intestinal CaBP (mol. wt. approximately 30,000) was purified by a new simplified procedure. The duodenal mucosa was scraped, homogenized in 40 mM TRIS, pH 7.4, containing 80 mM NaCl, and centrifuged at 100,000 x g. The supernatant was then concentrated and subjected to ion exchange chromatography on SP-Sephadex (C-50). The column was equilibrated and eluted with 50 mM sodium acetate, pH 5.0. CaBP eluted off the column just after the void volume as demonstrated by the Chelex-⁴⁵Ca competitive binding assay. The sample was then concentrated and subjected to gel filtration on Sephadex G-100 which was equilibrated and eluted with the homogenizing buffer.

The CaBP was determined as being pure on the basis of disc gel (basic) and sodium dodecyl sulfate gel electrophoresis. Antiserum against CaBP was prepared in rabbits. The antiserum was then adsorbed against a rachitic chick intestinal preparation, which is void of the vitamin D dependent CaBP, as a further precaution to assure a pure antiserum against CaBP. The acquisition of the chick intestinal anti-CaBP antibody and the human kidney anti-CaBP antibody, previously prepared, lead to cross antigenicity studies. Homogenate supernatants (100,00 x g) from various animal tissues, to include intestinal and kidney preparations from human, chicken, pig, and rat, were spotted on double immunodiffusion Ouchterlony plates against the two antibodies. A line of identity suggested that all the antigenic sites on the one antigen were also found on the other. A partial line of identity demonstrated that some but not all of the antigenic sites on the one antigen were present on the other. The human kidney antiserum demonstrated cross reactivity with all the various preparations except the intestinal preparations from the pig and rat. Total lines of identity existed between the kidney preparations of human, pig, and rat; whereas only partial lines of identity existed between these and the chick preparations of intestine and kidney. Total line of identity existed between the intestine and kidney preparations of the chick. Only a partial line of identity existed between the human preparations of intestine and kidney and no line of identity existed between human intestine preparation and the chick intestine preparation. The chicken intestinal antiserum demonstrated cross reactivity with all the various preparations except the intestinal preparations of human, pig, and rat. Total lines of identity existed between the chick intestinal and kidney preparations. Only partial lines of identity existed between these and the kidney

preparations of human, pig, and rat. Total lines of identity existed among the human, pig, and rat kidney preparations. Secondary precipitin lines were present in some of the preparations. While using human kidney antiserum, the secondary precipitin lines showed up in the preparations of chick intestine, chick kidney, and human kidney. These secondary precipitin lines may be artifacts due to an impure antiserum. However, the chick gut antibody preparation is highly specific for vitamin D requiring proteins because it was adsorbed against a rachitic chick intestinal preparation. Therefore, a possibility exists that the tissues have more than one CaBP or at least a protein or peptide similar enough to be precipitated with the anti-CaBP antibody.

Monkey intestinal CaBP and pig intestinal CaBP (both approximately 13,000 mol. wt.) were also purified by the procedure described above for the chick intestinal CaBP. Rabbit antisera against these CaBP are now in preparation. These antibodies will be used to demonstrate cross reactivities between CaBP from various animal species and in determining similarities between different CaBP. Hopefully cross reactivity will exist between one of these antibodies and the human intestinal CaBP so that human gut biopsies can be analyzed for CaBP concentration.

A semi-quantitative method using double immunodiffusion Ouchterlony plates was used to measure CaBP concentrations. The sensitivity of detection was demonstrated by using purified chick intestinal CaBP and its antibody. A precipitin line could be identified with as little as 64 ng of the protein. The precipitin line could also be made more pronounced by dialyzing the plate against TRIS-saline, pH 7.4, and then subjecting it to a 30-minute bath in methanol: water:acetic acid (50:50:10). This method of detecting CaBP is roughly 50 to 100 times more sensitive than the Chelex-⁴⁵Ca competitive binding assay. However, the Chelex assay still has the advantage of being more quantitative in the measurement of CaBP. This double immunodiffusion method has been extremely helpful in demonstrating the time of CaBP onset in rachitic chicks given vitamin D or one of its active metabolites.

Immunohistochemical evidence has shown a CaBP in the cytoplasm of the pancreatic islet cell. The low level and the apparent instability of the protein in the pancreatic homogenates have frustrated attempts at its complete isolation and purification. However, addition of a serine proteinase inhibitor, phenylmethanesulfonyl flouride, to the homogenate has permitted the demonstration of a pancreatic CaBP in heat treated concentrated preparations. Double immunodiffusion was used to detect CaBP with antibody against chick gut CaBP. The following approaches were used to compare the chemical natures of pancreatic and intestinal CaBP in chick. (1) Radial immunodiffusion established a line of identity between the two proteins which suggests that all antigenic sites on the intestinal

CaBP are also present on the pancreatic CaBP. (2) Immuno-electrophoresis revealed precipitin lines in comparable locations. (3) Cationic exchange filtration on SP-Sephadex (C-50) and gel filtration on Sephadex G-100 were similar. (4) Thin layer isoelectric focusing demonstrated that both proteins have an isoelectric point of approximately 4.5. Thus CaBP in chick pancreas is not only antigenically identical to that in the gut but also its chemical nature (pI approximately 4.5) and its physical nature (mol. wt. approximately 30,000) are similar.

CONCLUSIONS:

1. CaBP can be purified from animal tissues more reliably and with less loss of activity by the new methods developed in the current fiscal year. The methods have already been applied to prepare CaBP from monkey and pig intestine, both of which are more similar to human intestinal CaBP than chick and thus more likely to provide antiserum that can be used for assay of human intestinal CaBP.
2. Cross antigenicity exists between CaBP from various animal species and tissues.
3. The improved double immunodiffusion technique is 50 to 100 times more sensitive than Chelex-⁴⁵Ca competitive binding assay and capable of detecting as little as 64 ng of CaBP.
4. The CaBP of chick intestine and pancreas is similar in antigenic, chemical (pI approximately 4.5) and physical (mol. wt. approximately 30,000) properties.

RECOMMENDATION:

The study should be continued.

PUBLICATIONS:

1. Zolock, D. T. and R. L. Morrissey. Calcium binding protein in chick pancreas which is immunologically similar to that found in chick gut. Fed. Proc. 35(7):1609, 1976 (Abstract).

STUDY NO. 9

Kinetics and Localization of CaBP
Appearance in Chick Intestine
Following 1,25-Dihydroxycholecalciferol Administration

PROBLEM.

Deficiency of calcium is one of the three most common nutritional

deficiencies observed in the American diet. In contrast, dietary phosphorus is usually adequate or excessive. Thus, a dietary imbalance of the calcium:phosphorus ratio is frequently observed. The degree of imbalance ranges from estimates of 0.25 in certain segments of the civilian population to the measured 0.59-0.8 observed in military nutrition surveys conducted by this laboratory. This is in contrast to the National Research Council (NRC) recommended ratio in the diets of most animal species ranging from 1.0 to 2.0. The NRC recommended ratio is 1.0 for the adult human and 1.50 for the growing child. It is probable that these dietary imbalances are related to the expenditure of \$6,448,298.00 required to operate Army dental facilities during FY 75. In the facilities, 859,772 tooth restorations were performed during that period. Such dietary imbalances could also influence the incidence of bone and joint injuries, which account for 25% of the operations performed in combat zone surgical hospitals. At current hospitalization cost (\$138.15/day) and a conservative estimate of \$20.00/day for soldier compensation, the bone and joint injuries treated in the Army between 1964 and 1969 would cost a total of \$768,877,354.70.

The only specific protein in which the concentration is known to correlate with the intestine's ability to transport calcium is calcium binding protein (CaBP). Thus, this study was undertaken to determine the time sequence for the appearance of CaBP in rachitic chick (CaBP totally absent) after repletion with 1,25-dihydroxycholecalciferol (the metabolically active metabolite of vitamin D). Detailed knowledge of which intestinal cell synthesizes CaBP and where it is localized within those cells during the acute phase of rickets healing would bring us closer to an understanding of the possible function of the protein and provide a basis for studies to determine the factors controlling its cellular concentration. Knowing these factors more precisely should enable us to intervene more knowledgeably with nutritional and therapeutic means of preventing or treating disease and injury.

RESULTS AND DISCUSSION OF RESULTS:

At various times after intraperitoneal injection with tritiated thymidine and oral administration of 1 µg (40 IU) of 1,25-dihydroxy vitamin D, chicks were sacrificed and evaluated to determine immunohistochemically the localization of CaBP as well as to assess functional capacity by several biochemical parameters. CaBP first appeared at 6 hours, at which time it was only present as a distinct band of staining at the base of intestinal villi. It was present in intestinal villus cells and absent in goblet cells. As time progressed, the band of staining widened until after 18 hours, CaBP was found throughout the villus tip. Intracellularly, CaBP was found both in the nucleus and in the cytoplasm, with nucleus staining being more prevalent in the base of the villi and cytoplasm staining being more prevalent near the tip. These results indicate that CaBP is synthesized by the intestinal absorptive cell itself and that induction

of synthesis is not uniform among absorptive cells. The ease of induction may be related to the "age" of the intestinal cell. Specimens are being evaluated by autoradiography to determine with certainty if this speculation has a factual basis. Several biochemical and other functional parameters of the gut's ability to transport calcium have been evaluated. RNA polymerase activity was elevated within 2 hours, peaked at 4 hours, and remained high during the first 24 hours after treatment. The in vitro accumulation of calcium by gut obtained at biopsy was elevated at 2 hours, peaked between 4 and 12 hours, and returned to the rachitic level between 18 and 24 hours. The in vitro inorganic phosphate accumulation was elevated at 6 hours, peaked at 8 hours and decreased between 18 and 24 hours. Serum calcium concentration was elevated at 4 hours and remained up through 24 hours. The in vivo transport of calcium was significantly elevated with 2.5 hours and continued to rise for at least 9 hours after treatment. The earliest time that CaBP has been detected in the mucosa (by using the double immunodiffusion technique, sensitivity about 64 ng CaBP) has been 4 hours.

Additional similar studies employing metabolic inhibitors have demonstrated that induction of CaBP is not blocked by actinomycin D, but it is blocked by cyclohexamide. Intestinal calcium transport is not blocked in animals treated with 1,25-Dihydroxy vitamin D and cyclohexamide in spite of their deficiency of CaBP. As a result of these findings, experiments have been initiated employing a wheat germ system to measure directly the presence or absence of mRNA in rachitic and 1,25-dihydroxy vitamin D treated chicks.

Although it remains unknown as to how CaBP is related to the intestine's ability to transport calcium, results of experiments in the current fiscal year have radically altered the traditional concept of potential CaBP functions. For example, CaBP has been and still is viewed by many to be a protein secreted by goblet cells and its function was to attract calcium from the lumen of the intestine to the intestinal cells for transport. The present findings would suggest that it is an intracellular protein associated with the cytoplasm, membranes and occasionally the nucleus of the intestinal absorptive cell and is not found in the goblet cell. Also, its necessity for the transport of calcium is questioned. Thus, experiments in progress will explore the possibility of a CaBP function in the protection of intracellular organelles during the rapid transport of high loads of calcium and/or as a protein induced by intracellular calcium which is somehow involved in feedback control of nuclear gene expression and thereby critical to the control of a cell's ability to transport calcium.

CONCLUSIONS:

Although it is still probable that CaBP is intimately related to the

long-term steady state ability of the intestinal absorptive cells to transport calcium and adapt to dietary calcium levels, it appears from recent studies that it may not be a transport protein which fully accounts for the effect of vitamin D. The results suggest that the initial effect of 1,25-dihydroxy vitamin D is some unknown phenomenon which increases the ability of the cell to transport calcium and that CaBP induction follows at a later time.

RECOMMENDATIONS:

Recommend that additional experiments and studies be developed to determine the function of CaBP and other factors involved in the control of the absorption and retention of calcium.

PUBLICATIONS:

1. Morrissey, R. L., R. N. Empson, Jr., T. J. Bucci, and D. D. Bikle. Immunoenzymatic localization of calcium binding protein (CaBP) in duodenal epithelium of rachitic and vitamin D repleted chicks. Fed. Proc. 35(3):339, 1976 (Abstract).
2. Ziporin, Z. Z., P. P. Waring, R. L. Morrissey and M. E. Lysne. The effect of vitamin D and dietary content of calcium and phosphorus on protein synthesis in rat duodenal mucosa. (Cleared by the Publication Review Committee and awaiting Commander's approval for publication as a Numbered Laboratory Report.)
3. Morrissey, R. L., R. M. Cohn, R. N. Empson, H. L. Greene, O. D. Taunton and Z. Z. Ziporin. Relative toxicity and metabolic effects of cholecalciferol and 25-hydroxycholecalciferol. (Submitted for publication)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H.Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71P		01		045	
b. CONTRIBUTING							
EXCLUDED FROM INDEX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Essentiality and Functions of Minerals in Military Nutrition							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry; 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 12		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
EXPIRATION:				76		1.9	
d. NUMBER: Not Applicable				FISCAL YEAR		37	
c. TYPE:				CURRENT		0.5	
e. KIND OF AWARD:				77		11	
f. CUM. AMT.				0		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: Letterman Army Institute of Research Biochemistry Division			
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Milne, D.B., DAC			
TELEPHONE: 415-561-3600				TELEPHONE:			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Sauberlich, H.E., DAC			
				NAME:			
				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Laboratory Animals; (U) Nutrition; (U) Military Nutrition; (U) Minerals; (U) Trace Elements; (U) Nutritional Requirements; (U) Nutrition Disorders							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Fundamental research is directed towards present or potential military problems concerning mineral nutrition and metabolism in health and disease. Various minerals have been implicated in wound healing, bone calcification and integrity, prevention of various cardiovascular and degenerative diseases, protein synthesis, and lipid and cholesterol metabolism. Techniques will be developed and investigations conducted that will provide knowledge as to the requirements, metabolism, utilization and functions of minerals that may be utilized in applied studies in military medicine and ration development. The influence of changes in food sources and processing on the level and availability of minerals in military diets and rations would be evaluated.</p> <p>24. (U) Through the use of laboratory animals, studies will be conducted on the essentiality, metabolism and biological function of various mineral elements. Also, factors influencing the availability and balance of the mineral elements will be studied.</p> <p>25. (U) 76 04 - 76 09 A sterile isolation system has been established for studies on the essentiality and functions of trace elements. A marginal silicon deficiency has been produced in rats. Silicon is required for normal growth and appears to influence calcium metabolism. Publications: Milne, D.B. A Method for Silicon Analysis in Biological Materials Utilizing Flameless Atomic Absorption Spectroscopy. Fed. Proc. 35:683, 1976 (Abstract). This work unit is being terminated.</p>							

ABSTRACT

PROJECT NO.	3A161102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biomedical Sciences
WORK UNIT NO.	045	Essentiality and Functions of Minerals in Military Medicine

The following investigation has been conducted under this work unit:

STUDY NO. 1 Essentiality and Functions of Silicon

A trace element sterile isolation system that excludes glass, metal, rubber, and dust has been set up to study the essentiality functions of silicon, a recently discovered essential trace element. Silicon is required for normal growth and is apparently associated with calcium and connective tissue metabolism. This implies a possible role in wound healing which could be important in military medicine.

BODY OF REPORT

WORK UNIT NO. 045

Essentiality and Functions of Minerals in
Military Nutrition

STUDY NO. 1

Essentiality and Functions of Silicon

PROBLEM:

Recently, silicon has been found to be essential for growing chickens and rats. Although it is the second most abundant element in the biosphere, little is known about its function and metabolism. Of particular military interest is the implication that silicon is involved with bone calcification and connective tissue metabolism, thus it may play an important role in wound healing. The initial experiments under this study were designed to establish procedures for studying silicon metabolism and to obtain basic information regarding silicon distribution and functions.

RESULTS AND DISCUSSION OF THE RESULTS:

A "trace element sterile" isolation system has been set up and tested for use in studying silicon function and metabolism. In this all plastic system, the animals do not come in contact with glass, metal, rubber, dust, or caretaker personnel. A marginal silicon deficiency was produced in rats in the initial experiment over a 32-day period by feeding them a purified amino acid basal diet which contained less than 5 ppm silicon. Addition of 500 ppm Si as $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ or 100 ppm Si as diphenylsilanediol significantly ($P < .025$) increased growth rates by 12.5 and 14.5% respectively. Tissues taken from all groups are currently being analyzed for silicon.

In a preliminary experiment, it was noted that the calcium binding activity in the duodenum was increased in rats maintained on a low silicon diet. This trend appeared to be reversed in the silicon supplemented animals. The results indicated that silicon may influence the normal absorption of calcium from the diet. Calcium binding activity and calcium absorption rates are currently being evaluated in rats that have been maintained on a low silicon diet in isolation as compared with rats on diets supplemented with silicon in a follow-up study.

CONCLUSIONS:

It was confirmed that silicon is essential for growth in the rat and appears to play a role in calcium metabolism.

RECOMMENDATIONS:

1. Studies on the mechanisms of silicon action on calcium metabolism and bone formation should be continued.
2. The effects of diet and age on the silicon content of the collagen fractions in various tissues should be investigated.
3. Evaluation of various procedures for the study of the metabolism and functions of silicon and other essential trace elements should be continued.

PUBLICATIONS:

Milne, D.B. A method for silicon analysis in biological materials utilizing flameless atomic absorption spectroscopy. Fed. Proc. 35:683, 1976 (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DDB'S INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71P		01		048	
b. CONTRIBUTING							
c. XEROX NUMBER	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) The Requirements and Metabolism of Essential Minerals in Man and Animals							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 01		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATES/EFFECTIVE:				b. PROFESSIONAL MAN YRS			
d. NUMBER ^a Not Applicable				c. FUNDS (In thousands)			
c. TYPE:				76 1.8 61			
e. KIND OF AWARD:				77 0.5 13			
f. CUM. AMT.				77 0 0			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME ^a Letterman Army Institute of Research Bioenergetics Division			
ADDRESS ^a				ADDRESS ^a Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME ^a Johnson, H.L., DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5066			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Charles, M.A., LTC, MC			
				NAME: Consolazio, C.F., DAC POC:DA			
22. KEY WORDS (Precede EACH with Security Classification Code)							
(U) Laboratory Animals; (U) Human Mineral Balances; (U) Trace Mineral Requirements; (U) Selenium Hg and Vitamin E Interrelationships - Minerals in Sweat							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To study the interactions among macro and trace elements as influenced by stresses similar to those encountered in combat (i.e., dehydration, nutrition and environment). To determine the requirements for trace minerals as affected by the dietary levels of other nutrients with emphasis on those minerals which may be marginal in present and future rations. To further elucidate the metabolism of selenium, vanadium, and other trace minerals.</p> <p>24. (U) The analyses of zinc contents of the sweat, urine and fecal samples collected during a 1972 electrolyte study are partially completed. Food, urine, sweat, and fecal samples were collected during two human studies to determine protein requirement in 1976; and the analysis of these samples for sodium, potassium, calcium, and magnesium is progressing. A protocol to study whole body retention of vanadium has been prepared and the study will be initiated.</p> <p>25. (U) 75 07 - 76 09. Diabetic rats have defective intestinal calcium transport and the defect may involve calcium binding protein. Various physiological parameters were evaluated in diabetic rats to study anomalies of calcium binding protein. It was demonstrated that diabetic rats have calciuria, phosphaturia associated with hypocalcemia, normophosphatemia and impaired calcium transport even in the presence of calcium binding protein.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biomedical Sciences
WORK UNIT NO.	048	The Requirements and Metabolism of Essential Minerals in Man and Animals

The following investigations have been conducted under this work unit:

- | | |
|-------------|--|
| STUDY NO. 2 | Regulatory Mechanisms of Calcium Binding Protein (CaBP) in Polar and Apolar Cells. |
| STUDY NO. 3 | Sweat Losses of Minerals and Their Effects Upon Balances and Requirements of Man Undergoing Physiological Studies. |
| STUDY NO. 4 | Whole Body Retention and Tissue Distribution of ⁴⁸ V in the Rat. |

Study No. 2. Diabetic rats have defective intestinal calcium transport and this defect may involve calcium binding protein. Various physiological parameters were evaluated in diabetic rats to study anomalies in calcium binding protein. It was demonstrated that diabetic rats have elevated serum alkaline phosphatase activity as associated with hypocalcemia, normophosphatemia, and impaired calcium transport, even in the presence of calcium binding protein.

Study No. 3. Food, urine, sweat, and fecal samples were collected during the 1972 Electrolyte Study and were available for further analyses of trace elements. Zinc analyses of all urines and sweat samples, and copper analyses of the sweat samples have been completed. Two 30-day studies on protein requirements during physical training have been completed and include sample collections for mineral balance studies. Analyses for sodium, potassium, calcium, and magnesium in these samples are progressing.

Study No. 4. A protocol has been developed to study the whole body retention and organ distribution of radioactive vanadium in rats fed a low vanadium diet and a vanadium-supplemented diet. Although the role of vanadium may be obscure, its importance in the control of phospholipid and cholesterol metabolism may have significant implications in the nutritional status of military personnel.

BODY OF REPORT

WORK UNIT NO. 048

The Requirements and Metabolism of
Essential Minerals in Man and Animals

STUDY NO. 2

Regulatory Mechanisms of Calcium Binding Protein (CaBP) in Polar and Apolar Cells

PROBLEM:

Calcium is one of many critical ions required for body function. When calcium is not absorbed or excreted properly, serious disorders result. The most common cause of these abnormalities in this country is immobilization. Since many aspects of nutritional and metabolic dysfunction (both military and civilian populations) result in impaired calcium transport and endocrine responses, and since calcium metabolism may be regulated in part by calcium binding protein (CaBP), it would be of fundamental value to elucidate and compare relationships of CaBP in both transport and secretory tissues.

RESULTS AND DISCUSSION OF THE RESULTS:

Diabetic rats have defective intestinal calcium transport and this defect may involve CaBP. It was demonstrated that diabetic rats have elevated serum alkaline phosphatase activity associated with hypocalcemia, normophosphatemia, and impaired calcium transport, even in the presence of CaBP. Although calcium transport was observed to be impaired in in vitro gut loop studies, the levels of CaBP appeared to be normal in the duodenal mucosa.

CONCLUSIONS:

These results suggest that the diabetic rat is not a good model for the study of calcium binding protein as it relates to calcium transport. An unexpected finding is that diabetes may be associated with renal unresponsiveness to parathyroid hormone (whereas bones are responsive) which results in a syndrome of pseudohypoparathyroidism.

RECOMMENDATIONS:

None. (The study has been terminated).

PUBLICATIONS:

Charles, M.A., P.R. Tirunagaru, A. Valentine, R. Morrissey, and D. Zolock. Calcium metabolism in experimental diabetes. X International Diabetes Federation Congress, New Delhi, India, 31 Oct-5 Nov 1976 (Abstract, in press).

PROBLEM:

The human nutritional requirements for most of the minerals have not been firmly established. Moreover, the dermal and sweat contributions to the excretion of these elements have not been fully recognized. Most balance studies are conducted in a relatively comfortable environment without heavy physical activity, so that these dermal losses of minerals are generally ignored. However, military tactical situations do not occur under such ideal environmental conditions, and with large sweat volumes produced by man in a hot jungle or desert, dermal losses of various trace minerals may deplete body stores and create deficiencies with intakes that were considered adequate. Therefore, the sweat losses of these essential nutrients during extended periods of profuse sweating must be measured and considered in establishing requirements for military personnel. Human studies conducted at this laboratory provide the opportunities to study these losses, requiring only sample analyses and calculation of balances since the samples have already been collected for other purposes.

RESULTS AND DISCUSSION OF THE RESULTS:

a. Electrolyte Study. Samples were collected from six subjects throughout a 12-week study, which included sweat collections during physical work in a 95°F exercise room. Calculations of sodium, potassium, calcium, and magnesium balances, including dermal excretion, have been previously reported. Zinc analyses of sweat and urine, and copper analyses of sweat samples have been recently completed. Food and fecal analyses have been delayed because adequate facilities are not available at present.

b. Protein Study. Two 30-day studies of protein requirements of man during physical training and exercise were conducted this year. Analyses for sodium, potassium, calcium and magnesium contents of the sweat and urine samples are currently progressing.

CONCLUSIONS:

None.

RECOMMENDATIONS:

Analyses of all samples available for macro and trace elements should be completed. Mineral balances, including sweat losses, should be calculated and the contribution of these sweat losses to the requirements for the various essential minerals should be evaluated. This information

should be used in establishing mineral requirements for the military person and in evaluating the nutritional adequacy of his/her diets and rations.

PUBLICATIONS:

None.

STUDY NO. 4

Whole Body Retention and Tissue Distribution of ^{48}V in the Rat

PROBLEM:

As the nutritional essentiality of additional elements is being discovered and established, information on their biochemical roles and metabolisms is required in order to assess the nutritional status of military personnel and to assure the nutritional adequacy of the rations provided for these personnel under all conditions. Recent studies have provided preliminary evidence that vanadium may be essential in mammalian metabolism and that its deficiency in human diets may already exist. Although the role of vanadium may be obscure, its importance in the control of phospholipid and cholesterol metabolism may have significant implications on the nutritional status of the military person.

RESULTS AND DISCUSSION OF THE RESULTS:

The initial protocol to study whole body retention and organ distribution of a radioactive tracer dose of vanadium in rats maintained on two levels of dietary vanadium has been prepared and the study will be conducted when personnel are available.

CONCLUSIONS:

None.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6335	76 09 30	DD-DR&E(AR)636	
3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	1. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
		61102A		3A161102B71P		01	
11. CONTRIBUTING						WORK UNIT NUMBER	
						059	
XXXXXXXXXX		CARDS 114(f)					
12. TITLE (Precede with Security Classification Code) ^a							
(U) Basic Studies in Lipids (06)							
13. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry; 012900 Physiology; 002600 Biology							
14. START DATE		15. ESTIMATED COMPLETION DATE		16. FUNDING AGENCY		17. PERFORMANCE METHOD	
66 04		76 09		DA		C. In House	
18. CONTRACT GRANT				19. RESOURCES ESTIMATE		20. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				b. PRECEDING		c. FUNDS (in thousands)	
b. NUMBER ^a Not Applicable				76		2	
c. TYPE				FISCAL 7T		.5	
d. KIND OF AWARD:				YEAR 77		0	
e. AMOUNT:							
f. CUM. AMT.							
21. RESPONSIBLE DOD ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				Department of Medicine			
				ADDRESS ^a Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME ^a Herman, R. H., COL MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
23. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME:			
				POC: DA			
24. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Lipid Utilization by Muscle; (U) Carbohydrate Utilization by Muscle; (U) Muscle Function; Combat Soldier; (U) Clofibrate; (U) Bile Steroids; (U) Lab Animals; (U) Lipids							
25. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23.(U) Maximum muscular activity is essential for the effective performance of the combat soldier. Calcium is an essential mineral which is necessary for normal muscle contraction bone formation and central nervous system function. Calcium metabolism and absorption is regulated by vitamin D and its metabolites, which are lipid soluble hormones. Defects in the metabolism of vitamin D or in the target tissues (small intestine, muscle, bone) will lead to impaired absorption or utilization of calcium which in turn will impair muscle contraction and bone metabolism. It is possible that some of the inability of muscle and bone to utilize lipid and carbohydrate normally under conditions of stress are secondary to defects in calcium metabolism.							
24.(U) In order to determine the metabolism of vitamin D in man it would be convenient to be able to study normal human small intestinal mucosa <u>in vitro</u> . We are developing an <u>in vitro</u> system using rachitic chick duodenal mucosa incubated for varying times after the <u>in vivo</u> administration of vitamin D metabolites (1,25-dihydroxy vitamin D ₃ (1,25(OH) ₂ D ₃) and 1α-hydroxy vitamin D ₃ (1α(OH)D ₃)). The response of various vitamin D-mediated parameters can then be determined including duodenal calcium and phosphate uptake, calcium binding protein, alkaline phosphatase, and ribonucleic acid (RNA) polymerase. The effect of inhibitors such as actinomycin D and cycloheximide on such vitamin D-mediated parameters can be studied.							
25.(U) 75 06-76 09 1,25(OH) ₂ D ₃ and 1α(OH)D ₃ stimulate calcium transport in the rachitic chick duodenum many hours earlier than the increases seen in alkaline phosphatase activity and calcium binding protein concentration. 1,25(OH) ₂ D ₃ acts more quickly than 1α(OH)D ₃ to stimulate calcium uptake and calcium binding protein. Calcium binding protein appears first in cells at the base of the villus. The inhibitors did not block calcium transport. (See Endocrin. 98: 258, 1976 and Fed. Proceedings 35: 339, 1976).							

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	059	Basic Studies in Lipids

The following investigations have been conducted under this work unit:

STUDY NO. 1 The Effect of 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) and 1α -hydroxy vitamin D_3 ($1\alpha OH D_3$) on Calcium-activated ATPase, Calcium Binding Protein, and Calcium and Phosphate Uptake in the Chick Intestine.

STUDY NO. 10 The Role of Vitamin D in the Response of Muscle to Stress.

Study No. 1. We have investigated the biochemical mechanism by which $1,25(OH)_2D_3$, the naturally occurring biologically active metabolite of vitamin D, and its synthetic analog, $1\alpha OH D_3$, regulate calcium and phosphate transport across the gut. We have administered physiologic doses of $1\alpha OH D_3$ and $1,25(OH)_2D_3$ to rachitic chicks and observed the chronological order by which these substances stimulate RNA polymerase, alkaline phosphatase, calcium binding protein (CaBP), calcium accumulation in vitro and transport in vivo, phosphate accumulation in vitro, and serum calcium and phosphate.

Our observations suggest that:

- 1) CaBP and alkaline phosphatase are not required for the initial stimulation of calcium transport;
- 2) RNA and protein synthesis are not required for the initial stimulation of calcium transport;
- 3) Actinomycin D effects may not be secondary to the inhibition of RNA synthesis.

Study No. 10. This study was performed to determine if hypertrophy would occur to the same extent in rachitic animals as in vitamin D-treated animals with equivalent exercise. The right achilles tendon of rats raised on a rachitic diet was severed, and one week later the weights of the soleus and plantaris of both hind limbs were compared. The extent of muscular hypertrophy in the operated limb of rachitic rats was compared to results obtained in similarly treated rats receiving vitamin D supplementation before and after the operation. Although the vitamin D supplemented rats grew better and had much higher serum calcium levels, their muscles did not hypertrophy to a greater extent than those of the rachitic rats. We conclude that vitamin D is not essential to exercise-induced muscle hypertrophy.

BODY OF REPORT

WORK UNIT NO. 059

Basic Studies in Lipids

STUDY NO. 1.

The Effect of 1,25-dihydroxy vitamin D₃ (1,25(OH)₂D₃) and 1 α -hydroxy vitamin D₃ (1 α OH D₃) on Calcium-activated ATPase, Calcium Binding Protein, and Calcium and Phosphate Uptake in the Chick Intestine.

PROBLEM:

Two types of fractures occur with high frequency in the military population: 1) fractures due to impact (e.g. missile wounds, sudden blow to a bone), and 2) stress fracture (also called march or fatigue fracture). Both types of fractures cause considerable morbidity and prolonged if not permanent disability in the affected soldier. Recent advances in the understanding of the mechanism of action of vitamin D make it possible to investigate the potential role of vitamin D in the etiology and treatment of these bone problems. These animal studies represent the investigation of in vitro techniques which, if successful, will be applied to the study of patients with these and related types of bone disease. We have investigated the biochemical mechanism whereby 1,25(OH)₂D₃, the biologically active metabolite of vitamin D₃, and 1 α OH D₃ (the synthetic analog of 1,25(OH)₂D₃) regulate calcium and phosphate transport across the gut. We determined the chronological order by which these substances affected the following in rachitic chick intestine: RNA polymerase, alkaline phosphatase (alk Pase), calcium binding protein (CaBP), calcium accumulation and transport (Ca U), phosphate accumulation (Pi U), and serum calcium and phosphate concentrations. We determined which cells of the villus respond to 1,25(OH)₂D₃ as a function of time. We have determined the relationship between RNA and protein synthesis (e.g., CaBP and alk Pase synthesis) and 1,25(OH)₂D₃ stimulated calcium and phosphate transport across the gut.

RESULTS AND DISCUSSION OF THE RESULTS:

We consistently observed a longer time required for 1 α OH D₃ to cause an increase in CaBP and calcium uptake by the duodenal mucosa. The difference may reflect the requirement for conversion of 1 α OH D₃ to 1,25(OH)₂D₃ before it is capable of stimulating CaBP production and calcium accumulation.

Calcium uptake by mucosal biopsy specimens in vitro correlates well with calcium transport measured by in vivo techniques (isolated duodenal loops) for the first 8 to 12 hours after 1,25(OH)₂D₃ administration.

1,25(OH)₂D₃ and 1αOHD₃ have comparable time courses of action on rachitic chick gut as follows:

Parameter	Onset of Effect		Maximal Effect	
	1,25(OH) ₂ D ₃	1αOHD ₃	1,25(OH) ₂ D ₃	1αOHD ₃
	(hours)		(hours)	
RNA polymerase	2	1-2	4	1-8
alk Pase	4-6	4	12-24	24
CaBP	4	6	12-24	24
Ca U	2-4	8	4-12	24
Pi U	6	6	8	8-24
Serum Ca	2-4	2	8-24	8-12

Using an immunoperoxidase localization technique, we observed that CaBP was initially detected in the intestinal epithelial cells at the proximal end of the villus 6 to 8 hours after the administration of 1,25(OH)₂D₃ or 1αOHD₃. Double immunodiffusion of the intestinal mucosal cytosol detected CaBP 4 hours after 1,25(OH)₂D₃ administration but not until 6 hours after 1αOHD₃ administration. Within 24 hours after 1,25(OH)₂D₃ or 1αOHD₃, CaBP could be found in epithelial cells lining the whole villus. Concomitant labelling of these cells with ³H-thymidine demonstrated that this spread of CaBP occurred faster than cell migration along the villus. This indicated that all epithelial cells along the villus could accumulate CaBP following 1,25(OH)₂D₃ administration but that the cells just distal to the crypts make detectable amounts of CaBP sooner than the cells closer to the tip. There was a decreasing gradient of alkaline phosphatase activity from tip to crypt that was unchanged by 1,25-(OH)₂D₃, although the level of activity at any point along the gradient increased with time after 1,25(OH)₂D₃; i.e., 1,25(OH)₂D₃ stimulated alkaline phosphatase activity along the entire villus at the same time by approximately the same amount. This clearly differs from the action of 1,25(OH)₂D₃ on CaBP production.

If CaBP and alkaline phosphatase were necessary for calcium and phosphate transport, blocking their synthesis should block the effect of 1,25(OH)₂D₃ on calcium and phosphate transport. We examined the effects of the RNA and protein synthesis inhibitors, actinomycin D and cycloheximide, respectively, on these 1,25(OH)₂D₃ mediated processes. Actinomycin D inhibited RNA polymerase activity, stimulated alkaline phosphatase activity in a time and dose-dependent fashion and was additive to the effect of 1,25(OH)₂D₃, caused little, if any, inhibition of 1,25(OH)₂D₃-stimulated CaBP production, caused no inhibition of 1,25(OH)₂D₃-stimulated calcium transport as measured in vivo (paradoxically, the 1,25(OH)₂D₃-stimulated calcium uptake by mucosal biopsies as measured in vitro appears to be blocked), stimulated phosphate accumulation of mucosal biopsies in vitro which was

additive to the effect of $1,25(\text{OH})_2\text{D}_3$, inhibited the rise in serum calcium following $1,25(\text{OH})_2\text{D}_3$ administration and stimulated serum phosphate concentration independent of $1,25(\text{OH})_2\text{D}_3$. Cycloheximide inhibited $1,25(\text{OH})_2\text{D}_3$ stimulated protein synthesis, inhibited alkaline phosphatase activity in a dose dependent fashion that was not reversed by $1,25(\text{OH})_2\text{D}_3$, inhibited $1,25(\text{OH})_2\text{D}_3$ stimulated CaBP production, caused no inhibition of $1,25(\text{OH})_2\text{D}_3$ -stimulated calcium transport, and inhibited $1,25(\text{OH})_2\text{D}_3$ stimulated rise in serum calcium.

These results indicate the following:

1. RNA and protein synthesis are not required for the initial stimulation of calcium transport by $1,25(\text{OH})_2\text{D}_3$. The discordance between calcium transport measured in vivo and calcium uptake measured in vitro is not yet explicable.
2. CaBP production and alkaline phosphatase activity are not required for the initial $1,25(\text{OH})_2\text{D}_3$ stimulation of calcium transport.
3. Actinomycin D exerts an effect on intestinal alkaline phosphatase and phosphate uptake that resembles "super-induction" by actinomycin D of various enzymes in the liver.

CONCLUSIONS:

1. $1\alpha\text{OHD}_3$ stimulation of CaBP and calcium uptake lags behind that of $1,25(\text{OH})_2\text{D}_3$ which suggests conversion of $1\alpha\text{OHD}_3$ to $1,25(\text{OH})_2\text{D}_3$ before this synthetic analog exerts its effects.
2. Stimulation of CaBP and alkaline phosphatase by $1,25(\text{OH})_2\text{D}_3$ does not occur in parallel in the same intestinal epithelial cells.
3. RNA and protein synthesis, including CaBP and alkaline phosphatase synthesis, are not required for the initial stimulation by $1,25(\text{OH})_2\text{D}_3$ of calcium transport across the gut.

RECOMMENDATIONS:

Work in the next year should be directed at the following questions:

1. Does $1\alpha\text{OHD}_3$ require conversion to $1,25(\text{OH})_2\text{D}_3$ for some or all of its effects?
2. What is the relationship between calcium transport, CaBP and alkaline phosphatase?

3. How does $1,25(\text{OH})_2\text{D}_3$ stimulate calcium and phosphate transport?
4. What is the mechanism whereby actinomycin D stimulates alkaline phosphatase activity and phosphate uptake?
5. Why does actinomycin D affect calcium transport differently than calcium uptake?

PUBLICATIONS:

1. Bikle, D.D., R.N. Empson, R.L. Morrissey, D.T. Zolock, R.H. Herman and M.M. Pechet. Sequential changes in the rachitic chick following $1\alpha\text{OHD}_3$ treatment. Endocrinology 98, (suppl.) 258, 1976 (abstract).
2. Morrissey, R.L., R.N. Empson, Jr., T.J. Bucci and D.D. Bikle. Immunoenzymatic localization of calcium binding protein in duodenal epithelium of rachitic and vitamin D-repleted chicks. Fed. Proc. 35: 339, 1976 (abstract).
3. Bikle, D.D. and H.R. Rasmussen. Effect of ions on $25-(\text{OH})\text{D}_3$ 1α -hydroxylase in isolated rachitic chick renal mitochondria. Fed. Proc. 35: 340, 1976 (abstract).
4. Rasmussen, H and D. D. Bikle. Calcium and non-vesicular secretion in the kidney: Calcium and mitochondrial functions. In: Calcium Transport in Contraction and Secretion. Ed. E. Carafoli. North Holland Publ. Co., 1975, pp 111-121.
5. Bikle, D.D., E.W. Murphy and H. Rasmussen. The ionic control of $1,25$ -dihydroxyvitamin D_3 synthesis in isolated chick renal mitochondria: the role of potassium. Biochim. Biophys. Acta. 437: 394, 1976.

STUDY No. 10.

The Role of Vitamin D in the
Response of Muscle to Exercise.

PROBLEM:

Vitamin D is thought to play a role in the recovery of the musculo-skeletal system from injury. Vitamin D may also be essential for the proper conditioning of the musculoskeletal system during training. We wished to determine whether muscle in a vitamin D-deficient animal responds to exercise in the same fashion as that in a normal animal using the rat model developed by Goldberg et al. (Med. Sci. Sports 7: 185, 1975).

We divided 36 weanling rats raised on a rachitic diet into three groups. Group A received no vitamin D, group B received vitamin D 1 day before surgery and during the subsequent week, and group C received vitamin D for 10 days before surgery and during the subsequent week. Surgery consisted of cutting the right achilles tendon. One week later, the animals were killed and the weights of the right plantaris and soleus muscles were compared to those of the unoperated left hind leg.

RESULTS AND DISCUSSION OF THE RESULTS:

The table summarizes the results of the study.

Group	Vitamin D	% Change		Serum Ca (mg/dl)	Body Weight (g)
		Plantaris	Soleus		
A	0	12.1 \pm 4	34.7 \pm 5	4.6 \pm .1	163 \pm 11
B	8 days	9.0 \pm 3	—	—	167 \pm 15
C	17 days	11.9 \pm 4	35.9 \pm 6	9.2 \pm .2	203 \pm 9

The rachitic rat muscle hypertrophied to the same extent as the vitamin D supplemented rat muscle. The pronounced differences in serum calcium concentrations and the increased body weight of the vitamin D supplemented animals attests to the efficacy of the vitamin D supplementation on these other parameters.

CONCLUSIONS:

Rachitic rats do not require vitamin D for muscle hypertrophy.

RECOMMENDATIONS:

Terminate this study.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY 76 04 02	4. KIND OF SUMMARY H.Termination	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING NA	8. DISSEM INSTRN NL	9a. SPECIFIC DATA- CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER		
a. PRIMARY	61102A	3A161102B71P	01		060		
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code)* (U) Basic Studies of Nutrition and Metabolism							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 002300 Biochemistry							
13. START DATE 66 07		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT a. DATES/EFFECTIVE: b. NUMBER: Not Applicable c. TYPE: d. KIND OF AWARD:				18. RESOURCES ESTIMATE a. PROFESSIONAL MAN YRS b. FUNDS (in thousands)			
EXPIRATION:				76 PRECING 1.0 3			
d. AMOUNT:				7T 0.3 1			
f. CUM. AMT.				77 0 0			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS* RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415-561-3600				NAME* Letterman Army Institute of Research Biochemistry Division ADDRESS* Department of Nutrition Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME* Milne, D.B., DAC TELEPHONE: 415-561-5872 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: Schnakenberg, D.D., MAJ, MS NAME: POC:DA			
21. GENERAL USE Foreign Intelligence Not Applicable							
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Nutrition; (U) Military Rations (Nutrients); (U) Mineral Metabolism; (U) Proteins; (U) Lipids; (U) Carbohydrates; (U) Nutrition							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) Fundamental research is directed towards present or potential military problems concerning nutrition and metabolism and the role of diet in health and disease. Environmental factors, such as altitude and cold, have a marked effect on nutrition and metabolism. Because of the military implications of such adverse effects, an understanding of the effect and means of avoiding or correcting the conditions is a major objective of the investigations. Techniques will be developed and investigations conducted that will provide knowledge as to the metabolism, utilization or functions of dietary nutrients that may be utilized in applied studies in military ration development and troop feeding. 24. (U) The significance of dietary and environmental interactions and their relevancy to human health and the adequacy of military rations will be studied. The effect of diet, altitude, cold, exercise and stress on protein metabolism will be determined through the use of defined diets and radioactively labeled amino acids. Alteration in subcellular components necessary for protein synthesis as related to dietary composition will be investigated. Experiments will be developed with laboratory animals to study the influence of minerals and their interaction with other dietary nutrients on promoting the healing of bone injuries as may be sustained in combat or on the prevention of renal calculi as occurs in troops in a hot climate. 25. (U) 75 07 - 76 09 Various semi-purified and purified diets have been evaluated for use in animal studies and appear to be adequate for short-term studies utilizing rats. This work unit is being terminated.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 66PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65
AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biomedical Sciences
WORK UNIT NO.	060	Basic Studies in Nutrition and Metabolism

The following investigation was conducted under this work unit:

STUDY NO. 4 Evaluation of Proposed Standardized Semi-
purified and Purified Diets for the Laboratory
Rat

Standardized casein and amino acid basal diets, with optimal vitamin and mineral mixtures, were evaluated as to their potential for use in animal studies. The diets tested appeared to be adequate for short-term rat studies. No significant differences were observed between the proposed diet and two commercial laboratory rat chows with regard to growth, hemoglobin levels, or hematocrits after a 110-day feeding trial. These diets can be used for further studies on the metabolism, utilization or functions of dietary nutrients.

BODY OF REPORT

WORK UNIT NO. 060

Basic Studies in Nutrition and
Metabolism

STUDY NO. 4

Evaluation of Proposed Standardized
Semipurified and Purified Diets for
the Laboratory Rat

PROBLEM:

A wide variety of laboratory animal diets which varied considerably in nutrient content have been in use in this and other research laboratories. Thus, difficulties have been encountered in comparing data from various studies. This study was designed to evaluate and standardize purified diets for use in studies on the metabolism, utilization or functions of dietary nutrients.

RESULTS AND DISCUSSION OF THE RESULTS:

Optimal mineral and vitamin mixtures for rat diets were proposed by members of the Department of Nutrition. These mixtures were tested by comparing two proposed semipurified casein diets and four proposed amino acid diets (containing different amino acid mixtures as reported in the literature) with commercial laboratory chows. Weanling rats were maintained on these diets for 16 weeks with growth rate and food intake monitored twice weekly. At the conclusion of the study the rats were sacrificed and blood taken for hemoglobin and hematocrit measurements and for biochemical evaluation of vitamin status. Gastrointestinal tracts were retained for histopathological examination.

After 110 days of experiment, there were no significant differences in the total growth of rats fed with the proposed purified or semipurified diets or the commercial laboratory chows. Hemoglobin levels (average, 15.5 g/100 ml) and hematocrits (average, 43.1% packed cells) were normal in all groups. No significant findings were observed upon histopathological examination of the gastrointestinal tracts. Biochemical evaluation of the plasmas and sera for vitamin status are ongoing.

CONCLUSIONS:

All the diets tested appeared to be adequate for short-term rat studies.

RECOMMENDATIONS:

The proposed diets should be evaluated further for adequacy in long-term feeding experiments and in reproduction studies. The studies should be extended to species other than the rat.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISM ^a INST ^a N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71P		01		063	
b. CONTRIBUTING							
XXXXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Disease (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		76 09		DA		C. In House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER ^a Not Applicable				76		15	
c. TYPE:				7T		.3	
d. AMOUNT:				CURRENT		0	
e. KIND OF AWARD:				77		0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				ADDRESS ^a Department of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME ^a Herman, R. H., COL, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Vitamin Requirements of the Combat Soldier; (U) Red Blood Cell Enzymes; (U) Red Blood Cell Membrane; (U) Blood Cell Metabolism; (U) Folic Acid.							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23.(U) The diet of the combat soldier must provide sufficient amounts and types of vitamins in order to meet his requirements. A suitable assay system must be established to determine the level of dietary vitamins which will be optimum for the combat soldier. It is known that folic acid increases the activities of glycolytic enzymes and fructose diphosphatase in the jejunum and liver of man. Red blood cells (RBCs) contain an elaborate folic acid metabolizing system which corresponds to that found in the liver and jejunum. The RBC may be an ideal model in which to study the mechanism of action of folic acid on glycolytic enzymes and may prove to be a suitable assay system for assessing the optimum level of dietary folic acid.							
24.(U) Red blood cells will be incubated <u>in vitro</u> with various amounts and types of folate cofactors and glycolytic enzyme activities will be measured. Purine biosynthetic enzymes will be analyzed to establish the extent to which these cofactors influence purine biosynthesis in RBCs. The <u>in vivo</u> and <u>in vitro</u> effect of purines on RBC glycolytic enzyme activities will be assayed.							
25.(U) 75 06-76 10 Incubation of red cells <u>in vitro</u> with varying concentrations of folic acid gave inconsistent results. Incubation of red cell hemolysates with folic acid <u>in vitro</u> had no effect on glycolytic enzyme activities. Further exploratory studies were negative. The work unit is to be terminated.							

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biomedical Sciences
WORK UNIT	063	Haemopoietic Metabolism as Related to Nutrition Genetics and Metabolic Disease

The following investigations have been conducted under this work unit:

STUDY NO. 2. The effect of folic acid on glycolytic enzymes in human red blood cells.

Study No. 2. Studies done previously have shown that the glycolytic enzymes in red blood cells obtained from patients treated with folic acid increase in activity with time. Since the increase in activity required several days to occur, it was postulated that this increase was an effect of folic acid on developing red blood cells. Previous in vitro studies gave inconsistent results. It was not clear that folic acid could affect the glycolytic enzymes of red blood cells in vitro. Since there is evidence to suggest that the adult red blood cell is relatively impermeable to folic acid this would support the idea that folic acid affects red blood cells developing in the bone marrow. Further studies were conducted on red blood cells in vitro. Varying concentrations of folic acid incubated in vitro with red blood cell suspensions gave inconsistent results. Incubation of folic acid with red blood cell hemolysates demonstrated that there was no appreciable effect of folic acid on the glycolytic enzymes. The reason for the difficulty in obtaining reproducible results when intact red blood cells are incubated with folic acid is unclear. Many possibilities can be envisioned to explain these results. However, from a practical point of view it would not seem worth the time and effort to pursue these studies further. This work unit is being terminated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INSTR ^a	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3A762772A813				025	
B. CONTRIBUTING	61102A	3A161102B71P		03		025	
C. EXTENSION ^a CARDS (114f)							
11. TITLE (Precede with Security Classification Code) ^a (U) Biological Investigations in Prediction and Protection Against Coherent Radiation							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 009600 Masers and Lasers 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 12		Cont		DA		C In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE				PRECEDING		76 4.5	
B. NUMBER ^a Not Applicable				FISCAL		77 1.3	
C. TYPE				YEAR		CURRENT	
D. KIND OF AWARD				77		5.0	
E. AMOUNT						233	
F. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				ADDRESS ^a Non-Ionizing Radiation Division			
				Department of Biomedical Stress			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME ^a Beatrice, E. S., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3347			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Zwick, H., DAC			
				NAME: Randolph, D. I., DAC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Laser Safety; (U) Laser Hazard; (U) Eye Damage; (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The establishment of human permissible exposure levels from coherent radiation from military lasers is based upon subhuman research results. Variables involved in the extrapolation from biological research to "safe levels" are being investigated. These variables include the effects of wavelength, pulse duration, retinal sensitivity, and transmission of the ocular media on coherent and incoherent threshold levels.</p> <p>24. (U) Direct ophthalmoscopic observation of corneal and retinal exposure sites after low level irradiation will be analyzed by light and electron microscopic evaluation to determine possible tissue alterations produced by presently accepted "safe levels". Psychophysical testing of primates at and below these levels will result in correlation between ophthalmoscopic, histologic, and psychophysical threshold levels. Laser sources to be tested will include argon, krypton, ruby, carbon dioxide, neodymium and erbium. Single exposures, as well as repeated exposures will be studied by these techniques.</p> <p>25. (U) 76 04 - 76 09 Various electrophysiological and behavioral techniques have been established to evaluate the functional effects of laser alteration. Present results indicate that retinal damage processes other than those involved in thermal damage mechanisms are present and are typically masked by the gross thermal damage process. Several new techniques and some new parameters are presently being studied for visible, near IR and near UV laser sources. Behavioral evaluation of CO₂ radiation (10.6 μ) have shown that the rhesus monkey cornea is relatively insensitive to this irradiation. At a level ten times below current safe level, the animals respond to the thermal sensation on the skin and eyelids.</p>							

*Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66
AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161102B71P	Health Effects of Military Lasers
TASK NO.	03	Human Ecology
WORK UNIT NO.	025	Biological Investigations in Prediction and Protection Against Coherent Radiation

The following investigations have been conducted under this Work Unit:

STUDY NO. 1	Psychophysics of Visible Radiation
STUDY NO. 2	Behavioral Effects of Coherent Radiation

The objectives have been to investigate the functional effects of low level laser irradiation on visual function, to describe the mechanism operative at these levels, and to correlate behavioral responses to laser radiation in the task-oriented primate. The studies have resulted in further evaluation of the long term effects of visible and infrared laser radiation. These exposures are below the present "safe" level. Additional studies have been initiated to assimilate the "field" use of lasers. The implications of these results may effect the use of military low energy laser systems in one-sided field exercises. As data indicate, subtle persistent effects in the usual system for long periods after laser radiation, it may be necessary to reassess the current safe levels used in the military environment.

BODY OF REPORT

WORK UNIT NO. 025

Biological Investigations in
Prediction and Protection
Against Coherent Radiation

STUDY NO.

1

Psychophysics of Visible Radiation

PROBLEM:

The safe use of military laser systems is based upon the establishment of permissible exposure levels. These levels are based upon the results of experiments to determine the minimum acceptable criteria which may result in alteration of the individual's ability to "see" after radiation.

RESULTS AND DISCUSSIONS OF RESULTS:

Retinal Functional Studies: Our existing functional criteria used to evaluate low level laser irradiation were expanded. A method for measuring the effects of low level visible, near ultraviolet (UV), and near infrared (IR) laser irradiation was developed by using retinal electrophysiology. This technique permits the assessment of low level long-term exposures on retinal function. The effects of low level laser irradiation on retinal spectral sensitivity and dark adaptation are being assessed. Present data indicated that retinal processes are affected at irradiation levels well below those levels which produced gross morphological damaged criteria.

In collaboration with Dr. William Ham, Commonwealth University, an effort is being made to extend the damage action spectra for gross morphological criteria into the near UV. Spectral sensitivity measurements were made on four rhesus monkeys, each having one aphakic eye. In each case the absence of the lens increased the retinal sensitivity in the blue and near UV wave lengths by the amounts expected, which supports previously published in situ transmission measurements of the rhesus lens. Dr. Ham is now using one of these animals to determine threshold ophthalmoscopic levels in the blue and near UV region of the spectrum. At the completion of his exposure paradigm, this animal will be returned to our laboratory and, after exposure, spectral sensitivity data will be collected. Other animals trained in functional criteria and exposed in Dr. Ham's laboratory will eventually be sent here for additional electrophysiological and after exposure behavioral evaluation.

Several specific technique problems were resolved during this period regarding retinal electrophysiological criteria. Halothane anesthesia is no longer used in our rhesus preparation as preliminary electrophysiological data indicated that it produced permanent changes in spectral sensitivity and dark adaptation as measured by our techniques.

Barbiturate anesthesia (Nembutal) was found to be a successful substitute in situations where eye movements or drifts were minimal. In cases where eye movement activity is extensive, pancuronium-induced paralysis accompanied by artificial respiration was used. The doses for using pancuronium over extended and repeated times has been worked out and is now a standard laboratory procedure.

A signal averaging program to analyse the repetitive waveform used in our lock-in amplifier technique was developed and tested. This technique now allows inspection of the waveform used by the lock-in amplifier. Excellent correlation of spectral sensitivity for these two measures has been obtained.

Visual evoked potentials and electroretinograms were obtained on a rhesus monkey to evaluate the effects on the retina of intravitreal injections of chloroquine. This study was done with LTC Gardner and the data are being analyzed.

The effect of stroboscopic illumination upon human performance was studied in conjunction with field tests of xenon arc lamps at MASSTER, Fort Hood, TX. The data consisted of scores in a pursuit rotor task and showed little effects of frequency of stroboscopic illumination when task learning was taken into account.

Behavioral Studies. Several long-term animals are being maintained and periodically tested for spectral sensitivity. Follow-up of studies are planned on several of these animals. In one animal, electrophysiological spectral sensitivity measurements were compared in a low-level argon irradiated eye and normal eye. Difference between eyes was noted; this may indicate long-term (3 years) effects. Our facilities and laboratory moves are now completed; follow-up fundusoscopic, electrophysiological, and behavioral measures will be reinitiated in our long-term functional animals. Two new behavioral tasks are being initiated. The first is dynamic visual acuity which will aid in our understanding of the factors involved in recovery from foveal laser irradiation. Both the digital logic and the behavioral paradigm have been completed and presently three monkeys are being trained. When fully trained, both suprathreshold and subthreshold exposures will be evaluated and compared with previous data on static acuity measures.

In the second task, an effort is being made to determine the most sensitive indicator of overall retinal alteration. A pilot study has begun in which a rhesus monkey is being trained to respond to the just-noticeable-difference (JND) between the presence and absence of a Ronchi grating. The animal controls a set of circular wedges by manipulation of a toggle switch. A projector behind the wedges images the grating in a screen. Thus far, the animal's training has proceeded to the point where it is actually tracking, via the wedges, his JND for one

grating. He will learn to respond to several other gratings ranging from 10 lines/cm to 100 lines/cm, after which he will be exposed to various laser wavelengths at levels far below those shown to produce damage. Thresholds for his JNDs for each grating will then be a measure of the animal's ability to resolve fine lines, as opposed to previous efforts in which animals had been required to detect a gap in a Landolt C. This method will be used to study the dynamics of flash-blindness recovery, the effects of protective materials (filters) upon performance in a laser environment, the effects of background energy and wavelengths upon the ability of a rhesus to resolve differences in spacial frequency, and to provide much needed behavioral data on the effects of high energy xenon, mercury, and UV sources.

Techniques for measuring eye movement in rhesus subjects have been investigated with the collaboration of Dr. Eugene Kwatny (Krusen Institute, University of Pennsylvania). A system designed by Dr. Kwatny to measure the human electrooculogram (EOG) eye movement was adapted for rhesus. The EOG was studied in both anesthetized and task-oriented awake rhesus subjects. Drift and adaptation problems make this technique less desirable than a second technique that involves detection of infrared light reflected from the cornea. A circuit to detect these eye movements has been fabricated and is currently being modified to measure eye movements in either anesthetized or awake task-oriented rhesus subjects.

CONCLUSIONS: None

RECOMMENDATIONS:

The need to characterize more precisely the mechanisms responsible for low level functional alteration is of major importance. Specific techniques employed at the photochemical level must be initiated to determine what kinds of photochemical alteration underlie functional changes, and to explore the alterations which occur in electrical activity from single photoreceptors and other retinal neural elements. Emphasis must also be placed on the expansion of morphological criteria to include techniques that will characterize more precisely the lateral neural connections in the retina as well as the ultrastructure of the photoreceptors. Development of these areas will lead to a more complete understanding of damage processes underlying functional alterations and ultimately will lead to optimal safety standards.

PUBLICATIONS:

1. Zwick, H. and G. H. Holst. The effects of intense long wavelength irradiation of the red photoreceptor in pseudemys. Mod. Prob. Ophthalmol. 1976. (In Press).
2. Zwick, H. and D.O. Robbins. Functional criteria for visible laser irradiation. ARVO, 1976.

BODY OF REPORT

WORK UNIT NO.	025	Biological Investigations in Prediction and Protection Against Coherent Radiation
STUDY NO:	2	Behavioral Effects of Coherent Radiation

PROBLEM:

The establishment of a "safe" level for exposure to carbon dioxide (CO_2) laser radiation is presented in TB Med 279 as equivalent to irradiation from the sun during a "bright sunny day." The realization of the effect of being "warmed" by infrared radiation may cause individuals to avoid the heat source by blinking or body movement which may reduce or delay the completion of a critical mission. If the subhuman primate alters his training task at "safe" levels, then the human "safe" level may be "unsafe" in some circumstances thus the mission will not be completed or will be aborted. Thresholds for the behavioral task are being investigated to determine the differences between the "safe" level and the level at which the rhesus detects and avoids the radiation.

The problems under study are fourfold: (1) To determine the differential threshold sensitivity of the rhesus monkey cornea, lids, and surrounding tissues to CO_2 laser irradiation; (2) To determine the long-term physiological effects of repeated exposures to CO_2 laser radiation at levels presently assumed "safe" for human exposures; (3) To determine the relationship between sensitivity of the rhesus monkey to heat and irradiation predictions made by a bionathematical model of heat flow; and (4) To assess the thermal sensitivity of the cornea to elevations in temperature.

RESULTS AND DISCUSSION OF RESULTS:

A new and extensively modified operant conditioning technique has been developed in this laboratory for use with rhesus monkeys. In this method, a version of the conditioned suppression technique, each is trained to a baseline rate of responding using variable ratio liquid reinforcement (Tang). The animal's head is held in position and a 200 mw/cm^2 stimulus is directed into his open eye for 20 sec. This is immediately followed by a 2.9 ma shock to the leg. The difference in the rate of response during the 20 sec exposure and the rate of response in the 20 sec preceeding that exposure is then calculated. Findings on two rhesus monkeys show that a dose as low as 10 to 15 mw/cm^2 can be sensed at tissues surrounding the cornea. CO_2 radiation directed through a 4mm aperture to the center of the cornea has elicited borderline responses in the rhesus monkey. Preliminary indications are that

the responsiveness of the cornea is significantly less than that of surrounding tissues. This is in direct conflict with previously reported research results in humans. Comparison of the rhesus skin thermal sensitivity data with data published on sensation of warmth on the forehead indicates that the thermal sensitivity of the rhesus is close to that of humans for these tissues.

CONCLUSIONS:

The technique developed in this laboratory is an especially valuable method for determining thresholds for various sensory stimuli. The animal's ability to detect a stimulus of at least 10 mw/cm² on the skin, while no responses have been observed when the cornea alone has been exposed, is in direct conflict with reports which indicate that corneal sensitivity to heat was significantly higher than in the surrounding tissues. Furthermore, the "safe" level of CO₂ exposure has been set at 100 mw/cm² for extended (>1 sec) viewing times by TB Med 279, the ANSI Z136 Committee on Laser Standards, BRH, and AR 40-46. Our findings thus far indicate that while no changes have been observed in the corneas and surrounding tissues of our subjects, they will respond accurately to levels ten times lower than these standards.

RECOMMENDATIONS:

Repeated exposure of the corneal tissues to low level CO₂ radiation could result in swelling of the epithelial layers of this organ while not affecting behavior in the present paradigm. A contrast sensitivity experiment has been devised to determine the changes in contrast required for an animal to detect the lines of a Ronchi grating. This will enable us to measure accurately the resolving power of the retina before, during, and after low level CO₂ exposures. The assumption implicit in this technique is that swelling of the cornea, however slight, will produce increased light scatter in the ocular media. Therefore, an increase in background illumination to discern the Ronchi grating pattern will be required. To characterize the functional effects of laser irradiation in more detail, it will be necessary to do correlative studies of behavioral, electroretinographic, and evoked occipital potentials in rhesus monkeys. Planning for this critical experiment is now underway.

PUBLICATIONS:

1. Randolph, D.I. and B.E. Stuck. Sensitivity of the rhesus monkey cornea and surrounding tissues to heat produced by CO₂ radiation. Proceedings of the 1976 Army Science Conference. (In press).

2. Randolph, D.I., B.E. Stuck and H.B. Gardner. Data and evaluation of Project SATAN (Strobes against troops at night). (In revision).

3. Randolph, D.I. and B.E. Stuck. A technique for evaluating thermal sensitivity at the rhesus monkey eye and surrounding tissues. (To Commander).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 76 04 02	4. KIND OF SUMMARY H.Termination	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8A. DISB'N INSTR'N NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71R		02		058	
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code)* (U) Nutritional and Metabolic Adaptations and Interrelationships							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 002300 Biochemistry; 002600 Biology; 012900 Physiology							
13. START DATE 65 05		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		76	
b. NUMBER* Not Applicable				FISCAL		7T	
c. TYPE:				CURRENT		0	
d. AMOUNT:				77		0	
e. KIND OF AWARD:				f. CUM. AMT.		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS* RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415-561-3600				NAME* Letterman Army Institute of Research Biochemistry Division ADDRESS* Department of Nutrition Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution) NAME* Askew, E.W., CPT, MS TELEPHONE: 415-561-5872 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: Hecker, A.L., CPT, MS NAME: POC:DA			
21. GENERAL USE Foreign Intelligence Not Applicable							
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Diet; (U) Adaptation; (U) Enzymes; (U) Fatigue; (U) Military Performance; (U) Biochemistry-Muscle; (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) To study adaptive changes in energy metabolism and to identify potential dietary responsive rate-limiting steps in energy generation during activity. To determine the interaction of nutrition and stress in these adaptations. Obtain basic information on aspects of biochemistry, physiology, and nutrition as related to muscle functions that will ultimately allow the recommendation of an improved program of nutrition in order to improve the ability of the soldier to cope with various environmental and military situations. 24. (U) Physically conditioned rats were utilized in experiments to test time course of biochemical adaptation to increase energy demands; influence of dietary intake and activity on adipose tissue cellularity and lipolysis; effect of activity on mineral metabolism and absorption of nutrients from the gut; influence of dietary lysine deficiency on biochemical adaptation of energy metabolism. 25. (U) 75 07 - 76 09 Endurance training leads to increased sensitivity of adipose tissue to catecholamines, and alterations in the synthesis or degradation of cyclic AMP. Such metabolic alterations regulate the delivery of fatty acids to muscle during exercise. Physical exercise is associated with alterations in concentration of iron, zinc, copper and manganese in skeletal muscle, heart and liver. In addition, exercise stimulates oxidation of leucine and reduces leucine incorporation into cardiac proteins. Protein synthesis in skeletal muscle is not stimulated by physical exercise.							

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	058	Nutritional and Metabolic Adaptations and Interrelationships

The following investigations have been conducted under this work unit during the past year:

STUDY NO. 6	Dietary Control of Lipid Metabolism
STUDY NO. 8	Studies on Mineral Metabolism and Interactions
STUDY NO. 11	Effect of Endurance Training on Leucine Metabolism in Skeletal and Cardiac Muscle

Study No. 6. The mechanism, whereby fatty acid mobilization is increased during exercise, was investigated. Two theories were tested: a) reduced adipose tissue cell size by exercise increases the sensitivity of the cells to catecholamine stimulation, and b) changes in the rate of production or destruction of cyclic adenosine 3':5'-monophosphoric acid (cAMP) is responsible for the increased sensitivity. The results indicate that such adaptation is not due to changes in cell size or cyclic nucleotide production.

Study No. 8. Physical exercise induces significant changes in the tissue concentration of iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). The levels of these four elements in the skeletal muscle are increased in exercised rats. Heart Zn and Cu levels were increased in response to training, while liver Fe, Zn, and Cu increased but Mn decreased. Furthermore, white fibered muscles contain the lowest level of Fe, Zn, Cu and Mn.

Study No. 11. Rats subjected to physical exercise oxidize leucine at a faster rate than the corresponding sedentary controls. Exercise reduces leucine incorporation into cardiac muscle proteins but stimulates leucine incorporation into soluble fractions of the skeletal muscle. The rate of protein synthesis in the skeletal muscle appears to be fiber dependent. The results suggest that the increased muscular efficiency observed during endurance training is not associated with an increase in protein synthesis.

BODY OF REPORT

WORK UNIT NO. 058

Nutritional and Metabolic Adaptations and Interrelationships

STUDY NO. 6

Dietary Control of Lipid Metabolism

PROBLEM:

The objective of these studies reported within this work unit is to obtain basic information on aspects of biochemistry, physiology, and nutrition as related to muscle functions that will ultimately allow the recommendation of an improved program of nutrition in order to improve the ability of the soldier to cope with various environmental and military situations. Previous work under this study demonstrated that exercise training increases the turnover rate of adipose tissue fatty acids. The increased turnover rate is probably related to two earlier findings under this study: exercise training increases skeletal muscle fatty acid oxidation and fatty acid mobilization from adipose tissue. The supply of fatty acids to the muscle has been suggested by other workers as one of the rate limiting steps in lipid utilization. As part of the overall goal to understand the partitioning of the oxidation of fat and carbohydrate during exercise, we investigated the cellular mechanisms whereby adipose tissue of trained rats can respond to catecholamine stimulation by releasing increased amounts of free fatty acids (FFA) to the blood. We attempted to test two hypotheses: 1) that the response to training was merely the result of a reduction in size of adipose tissue cells and hence could be duplicated by making cells of untrained rats smaller by dietary energy restriction, and 2) that this adaptation of lipolysis to exercise training was due to increase cyclic adenosine 3':5'-monophosphoric acid (AMP) production by the adipose cells.

RESULTS AND DISCUSSION OF THE RESULTS:

Results of earlier studies, were confirmed: exercise trained rats had smaller adipose tissue cells and these cells possessed increased epinephrine stimulated lipolytic activity. Decreased cell size in food-restricted untrained rats was not accompanied by any change in the lipolytic rate within adipose tissue. Rats which trained at a lower than usual workload had adipose cells of similar size to rats exercising at a higher workload but they possessed lower lipolytic capabilities which suggests that exercise causes an enzymatic adaptation within the adipose cell that is unrelated to cell size but directly related to energy expenditure. Exercise training was associated with decreased tissue cyclic AMP levels in response to exercise training. This decrease was apparently the result of unchanged adenyl cyclase activity and enhanced phosphodiesterase activity. The change in the ratio of these two enzymes may indicate that adipose tissue cyclic AMP metabolism is more closely controlled and results in a

higher peak response to catecholamine stimulation by mechanisms yet unidentified, but the duration of this lipolytic response may be decreased because of cyclic AMP destruction. These observations may account for the modulation of plasma FFA overshoot which may be seen in trained athletes following the cessation of exercise compared to untrained individuals.

CONCLUSIONS:

The results of this study indicate that adipose tissue cells adapt to exercise training by decreasing the amount of lipid stored per cell but increasing the turnover of existing lipid stores. This increased turnover may be facilitated by adaptive mechanisms making fatty acids deposited in the cells of trained athletes more readily available during exercise. The mechanism whereby mobilization of lipids is enhanced by training does not appear to be a result of cellular surface area:volume considerations or an increase in catecholamine receptor sites. The feasibility of increasing lipid utilization during exercise by dietary treatments may depend upon the nature of the adaptation of lipolysis to exercise.

RECOMMENDATIONS:

Further studies should be conducted to determine the effects of physical exercise on adipose tissue lipolysis and fatty acid utilization by the skeletal muscle.

PUBLICATIONS:

1. Askew, E.W., G.L. Dohm and R.L. Huston. Fatty acid and ketone body metabolism in the rat: response to diet and exercise. *J. Nutr.* 105:1422, 1975.
2. Askew, E.W., H. Barakat, G.L. Kuhl and G.L. Dohm. Response of lipogenesis and fatty acid synthesis to physical training and exhaustive exercise in rats. *Lipids* 10:491, 1975.
3. Askew, E.W., R.L. Huston, C.G. Plopper and A.L. Hecker. Adipose tissue cellularity and lipolysis: response to exercise and cortisol treatment. *J. Clin. Invest.* 56:521, 1975.
4. Huston, R.L., P.C. Weiser, G.L. Dohm, E.W. Askew and J.B. Boyd. Effects of training exercise and diet on muscle glycolysis and liver gluconeogenesis. *Life Sci.* 17:369, 1975.
5. Askew, E.W., A.L. Hecker and F.B. Stifel. Effect of graded exercise and food restriction on adipose tissue cellularity and lipolysis. *Fed. Proc.* 35:760, 1976 (Abstract).
6. Askew, E.W. and A.L. Hecker. Adipose tissue cell size and lipolysis in the rat: response to exercise intensity and food restriction. *J. Nutr.* 106:1351, 1976.

7. Askew, E.W., A.L. Hecker and W.R. Wise, Jr. Dietary carnitine and adipose tissue turnover rate in exercise trained rats. Submitted for publication.

8. Askew, E.W., J.P. Brown and A.L. Hecker. Observations on preadipocyte distribution patterns in rat adipose tissue. Submitted for publication.

9. Askew, E.W., A.L. Hecker, V.G. Coppes, and F.B. Stifel. Cyclic AMP metabolism in adipose tissue of exercise trained rats. Submitted to the LAIR Manuscript Review Committee.

10. Askew, E.W., G.L. Dohm, P.C. Weiser, R.L. Huston, and W.H. Doub, Jr. Supplemental dietary carnitine and lipid metabolism in exercising rats. Submitted to the LAIR Manuscript Review Committee.

STUDY NO. 8 Studies on Mineral Metabolism and Interactions

PROBLEM:

The intimate involvement of several bioinorganic nutrients in intermediary metabolism suggests a possible important role in physical endurance. However, limited information exists on the trace mineral composition of muscle and other tissues, as influenced by cell type and function and by physical training. Thus, this study was conducted to determine the level of iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) in several tissues of the laboratory rat, and to examine the impact of endurance exercise on their concentration in these tissues.

RESULTS AND DISCUSSION OF THE RESULTS:

The concentration of four essential trace elements (Fe, Zn, Cu and Mn) were significantly altered in several rat tissues by physical exercise. Three posterior skeletal muscles known to differ markedly in fiber composition were examined. The levels of these four elements were all substantially greater in the soleus (composed of predominately red and intermediate fibers) than in the quadriceps (a mixed fiber muscle), while the psoas (a white fibered muscle) had the lowest levels. Training was associated with an increase in the concentration of all four minerals in the quadriceps, while the soleus exhibited an increase in Fe, Cu and Mn, and the psoas increased in Zn, Cu and Mn. The concentrations of Zn and Cu were significantly elevated in heart tissue and the levels of Fe, Zn and Cu were greater and Mn lower in the livers of endurance-exercised rats. Whole blood Cu levels were also increased in trained animals. The changes observed in these tissues were not the result of tissue hypertrophy or alterations in water content.

CONCLUSIONS:

The results of this study demonstrate another series of tissue physiochemical alterations associated with physical training. The substantial muscle mineral differences are probably related to the inherent biochemical and physiological dissimilarities in the fiber composition of the three muscles examined. The observed changes in mineral profile of skeletal and heart muscle are probably associated with the intracellular enzymatic adaptations to endurance exercise, of which these metallic micronutrients serve as essential constituents or cofactors. The liver alterations of these trace elements is more obscure since this organ serves as a substantial storage depot for these cations, while also requiring them for intracellular biochemical processes. The enhanced blood Cu level is probably a reflection of the previously reported increase in blood ceruloplasmin of exercised rats.

RECOMMENDATIONS:

A definite need exists for further research on the effect of endurance training and exhaustion upon the tissue concentration of several essential minerals. These studies should include possible alterations in dietary requirement for these elements due to physical training, an examination of the performance detriments and biochemical alterations associated with mild deficiencies and whether physical conditioning contributes to an increased turnover of tissue minerals. Studies of this type would contribute to the fundamental knowledge necessary for an adequate evaluation of the nutritional dietary adequacy of individuals exposed to heavy physical training.

PUBLICATIONS:

Kuhl, G.L., A.L. Hecker, E.W. Askew and J.E. Canham. Muscle minerals: effect of muscle type, physical training and exhaustion. Fed. Proc. 35:796, 1976 (Abstract).

STUDY NO. 11	Effect of Physical Training on Leucine Oxidation and incorporation into tissue Proteins
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PROBLEM:

Previous studies in this laboratory showed that muscle preparations from trained rats have a greater capacity to oxidize leucine than similar preparations from untrained animals. Enhanced leucine oxidation occurs early in training and becomes more pronounced with continued training. These observations indicate an increased utilization of leucine by trained animals and strongly suggest a marked effect of physical training on overall protein metabolism. Consequently, the present study was concerned with the effects of physical

training on in vivo leucine oxidation and incorporation into various subcellular or muscle proteins.

RESULTS AND DISCUSSION OF THE RESULTS:

Six-week-old male rats were subjected to an intensive twelve-week treadmill training program. A corresponding group of sedentary pair-fed animals were used as controls. The animals were allowed to rest 48 hours prior to experimentation. The data indicate that trained animals oxidized leucine at a faster rate than the controls. Furthermore, specific radioactivities of crude protein, mitochondrial or microsomal fractions of the cardiac tissue from trained rats were significantly lower than the radioactivities of the corresponding fractions from the controls. A similar trend was observed in the soleus and psoas muscles. In both the skeletal and cardiac muscles, the mitochondrial protein had a higher specific activity than either the crude homogenate or microsomal preparation. The rate of protein synthesis also appeared to be fiber dependent with the soleus (red and intermediate fibers) having a greater specific activity than the psoas (white fibers) muscle. In contrast, sarcoplasmic proteins in the gastrocnemius muscle from trained rats had a higher specific activity than those from the controls, suggesting an increased turnover of soluble proteins in the muscle of trained animals. No effect of training was observed on leucine incorporation into myofibrillar or stromal protein fractions.

CONCLUSIONS:

The present study demonstrates significant effects of endurance training on muscle protein metabolism. Protein synthesis in the cardiac muscle, as a result of training, appears to be decreased. In contrast, protein synthesis in the soluble fraction of the skeletal muscle is stimulated by physical exercise. The data also provide evidence that physical training significantly increases the catabolism of leucine. Consequently, it appears that physical training affects protein turnover in some organs or tissue fractions.

RECOMMENDATIONS:

Further studies concerned with basic interrelationships between endurance training and protein metabolism are needed. These should include effects of exercise on the leucine pool size in specific muscles and on protein turnover in such muscles. In addition, studies concerned with the utilization of leucine or other amino acids during a period of exercise should be initiated.

PUBLICATIONS:

1. Hecker, A.L., G.J. Klain, E.W. Askew and W.R. Wise, Jr. Adaptation to exercise: leucine metabolism in skeletal muscle. *Physiologist* 18:242, 1975 (Abstract).

2. Hecker, A.L., G.J. Klain and E.W. Askew. Effect of endurance training on leucine metabolism in skeletal and cardiac muscle. Fed. Proc. 35:528, 1976 (Abstract).

3. Dohm, G.L., A.L. Hecker, W.E. Brown, G.J. Klain, F.R. Puente, E.W. Askew and G.R. Beecher. Adaptation of protein metabolism to endurance training. Increased amino acid oxidation in response to training. Submitted to LAIR Manuscript Review Committee.

4. Dohm, G.L., G.R. Beecher, A.L. Hecker, F.R. Puente, G.J. Klain, E.W. Askew, and C.P. Smith. Adaptation of protein metabolism to endurance training. Changes in protein synthesis in response to training. Submitted to LAIR Manuscript Review Committee.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OA 6334	76 09 30	DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISB'D INSTR'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
76 04 02	1. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71R		02	
b. CONTRIBUTING		CARDS 114(F)				U62	
11. TITLE (Precede with Security Classification Code)* (U) Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet, and Steroids in Normal Man and Disease (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
003500 Clinical Medicine; 002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 11		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATES/EFFECTIVE:				a. PROFESSIONAL MAN YRS			
b. NUMBER: Not Applicable				b. FUNDS (In thousands)			
c. TYPE:				c. PRECEDING			
d. AMOUNT:				76 2 60			
e. KIND OF AWARD:				77 0 0			
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Herman, R. H., COL, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME: POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Laboratory animals; (U) Muscle Function of the Combat Soldier; (U) Muscle; (U) Metabolism; (U) Exercise; (U) Electrolytes; (U) Diet; (U) Steroids; (U) Myoglobin.							
23. (U) The successful outcome of military operations ultimately depends on the strength and endurance of the combat soldier. In order to maximize muscle strength and endurance, the metabolic processes of muscle, and those factors which enhance the development and maintenance of optimal muscle function must be studied. Such studies might lead to the identification of musculotropic agents, which in conjunction with physical training and essential nutritional substances would promote and maintain maximal muscle function.							
24. (U) Muscle tissue will be obtained from animals and patients with a variety of muscle diseases. Metmyoglobin reductase activity will be determined in these muscle tissue samples. The properties of the enzyme (in a crude homogenate) will be evaluated. Purification of the enzyme and definitive studies of the purified enzyme will be undertaken.							
25. (U) 75 06-76 09 Beef heart muscle contains specific metmyoglobin reducing activity. The rates of metmyoglobin reduction <u>in vitro</u> are far greater than any previously reported for non-enzymatic or non-specific systems. Preliminary attempts to purify the enzyme by standard methods have resulted in 250-fold purification (specific activities around 10,000). Instability and loss of enzyme activity during chromatography and storage can be prevented by the addition of 1 mM ethylene diaminetetraacetic acid. Hagler, L., R. I. Coppes, Jr., and R. H. Herman. Metmyoglobin reductase: Identification of a reduced nicotinamide adenine dinucleotide dependent enzyme from bovine heart which reduces metmyoglobin. Fed. Proceedings 35: 1423, 1976							

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Bio-Medical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.	062	Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet and Steroids in Normal Man and Disease

The following investigations have been conducted under this work unit:

STUDY NO. 1. Studies Concerning the Mechanism which Control the Redox State of Myoglobin

Studies have been performed which, for the first time, clearly demonstrate the presence of metmyoglobin (MetMb) reducing activity in muscle. The rates of reduction are far greater than any previously reported for non-enzymatic or non-specific systems. These preliminary studies were carried out using the soluble supernatant fraction of beef heart homogenate as the source of enzyme. The major emphasis this year has been an attempt to purify the enzyme in order to perform more detailed and more definitive studies. Attempts have been made to achieve purification by standard biochemical methods, including carboxymethyl and diethylaminoethyl Sephadex and cellulose column chromatography. These standard methods have yielded small amounts of enzyme with specific activities of around 10,000 which represents 250-fold purification. In the course of these experiments as enzyme purification proceeded, it was found that ethylenediamine tetracetic acid (EDTA) prevented loss of activity during chromatography and storage. Efforts in the immediate future will be directed at continuing large-scale purification of the enzyme.

BODY OF REPORT

WORK UNIT NO. 062

Muscle Metabolism as Related to Exercise, serum Electrolytes, Diet and Steroids in Normal Man and Disease

STUDY NO. 1

Studies Concerning the Mechanism which Control the Redox State of Myoglobin

PROBLEM:

Hemoglobin (Hb) and myoglobin (Mb) share a number of properties which include reversible oxygenation to form oxyhemoglobin (HbO₂) or oxymyoglobin (MbO₂); or irreversible oxidation to methemoglobin (MetHb) or metmyoglobin (MetMb), respectively. Whether these heme proteins undergo oxygenation or oxidation depends on a number of factors which are complex and incompletely understood. Under physiological conditions in vivo, only 2 to 3% of hemoglobin in red blood cells is in the met-form. Several efficient enzymatic systems have been described which continually reduce MetHb, thereby preventing its accumulation to any appreciable extent. The enzymes responsible for this reduction utilize NADH or NADPH, and in some cases require an electron carrier such as methylene blue for in vitro study. By far the most active system which requires ferrocyanide ion activation as described by Hegesh and Avron.

Much less attention has been given to the possible existence of similar systems which reduce MetMb. MetMb normally is not thought to be present in muscle in any appreciable quantity despite the greater susceptibility of Mb to oxidation than Hb. It is reasonable to assume that muscle must contain a highly active mechanism for MetMb reduction, otherwise the continued formation of MetMb would go unopposed. The presence of diaphorases in muscle is well known. However, the existence of a specific MetMb reductase, analogous to MetHb reductase activity in red blood cells has not been convincingly demonstrated heretofore.

Enzymatic reduction of MetMb by NADH and NADPH dependent mechanisms has been shown by Rossi-Fanelli et al, however, a specific MetMb reductase activity was not found. An enzyme which will reduce metmyoglobin has been described in dolphin muscle. Enzyme activity was demonstrable with either NADH or NADPH at pH 7.0 and required the presence of methylene blue. How this enzyme differed from diaphorase is not clear. Presumably enzymatic reducing activity has also been demonstrated in both intact and ground meat, but without clarification of the mechanism. Furthermore, Brown and Synder have shown efficient non-enzymatic MetMb reduction under suitable circumstances in vitro. Moreover, immunologic and electrophoretic studies have shown that ferrocyanide-activated MetHb reductase activity was detectable in several tissues including muscle.

Despite the failure of past investigators to demonstrate convincingly specific enzymatic MetMb reduction, it is logical to conclude that if Methb reductase exists in red blood cells, an analogous enzyme for MetMb reduction should exist in muscle. Initial studies (discussed in the 1975 Annual Report pp 101-105) demonstrated the presence of a specific NADH-dependent metmyoglobin reductase in the soluble supernatant fraction of homogenized beef heart. The optimum assay conditions and some of the properties of the enzyme (in the crude system) were established. The next series of studies and experiments were aimed at purifying the enzymatic reducing activity in the supernatant fraction of homogenized beef heart.

Muscle function is impaired in wounded soldiers by direct injury (trauma, muscle wounds, excessive exercise) and/or immobilization of limbs and/or bed rest. In order to facilitate healing and to reverse atrophy of muscle it is important to understand the mechanism involved in exercise-induced hypertrophy and the immobilization-induced atrophy of muscle. It is postulated that myoglobin is involved in these exercise-dependent responses of muscle via its function as an intracellular carrier of oxygen. Since oxygen also oxidizes myoglobin to the met-form it can be logically argued that there must exist a mechanism for the reduction of metmyoglobin. If so, one might expect also that defects in the metmyoglobin reduction system can lead to exercise-induced injury, diminished hypertrophy of muscle during exercise, accelerated atrophy during immobilization, and prolonged recovery after injury.

RESULTS AND DISCUSSION OF THE RESULTS:

A standardized approach to the purification procedure was taken. We attempted to elute enzyme-rich crude fractions from carboxymethyl Sephadex columns with ionic and pH gradients. These maneuvers were only partially successful since there were large losses of enzyme with each attempt. Similar maneuvers with DEAE Sephadex also resulted in large losses of enzyme. Chromatography on DEAE cellulose provided a greater yield of partially pure enzyme which lost activity rapidly over the next few days whether refrigerated or frozen. Ethylenediamine tetracetic acid (EDTA) protected the enzyme, not only during the column chromatography, but also in the resulting fractions which contain purified enzyme and a minimal amount of other protein. These fractions remain active and tolerate freezing and thawing without apparent loss. Thus, the addition of EDTA to the chromatography buffers provides the necessary protection to allow further enzyme purification. Initial chromatographic experiments which started with partially purified enzyme solution have yielded fractions with specific activities of over 10,000 (which represents 250-fold purification). Current efforts involve attempts to establish the most efficient and effective chromatographic system for large scale enzyme purification. Once this has been done, enzyme can be purified in quantities which are large enough to allow further definitive studies to be undertaken.

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CONCLUSIONS:

These studies have conclusively demonstrated for the first time the presence of a specific, NADH-dependent, metmyoglobin reductase in the soluble supernatant fraction of homogenized beef heart. Subsequent studies have shown that this enzymatic activity can be purified (at least 250-fold to date) and stored in the frozen state without loss of activity. An absolute requirement for EDTA in the purification scheme has been determined. Some of the column chromatographic conditions have been evaluated in anticipation of large-scale purification of the enzyme.

RECOMMENDATIONS:

These studies represent a continuation of previous investigations of muscle function, the biochemistry of myoglobin, and the primary and secondary myoglobinurias. Myoglobin provides the only oxygen reservoir in muscle. The unavailability of sufficient oxygen could lead to both structural and functional defects in the muscle. These studies should be continued.

PUBLICATIONS:

Hagler, L., R. I. Coppes, Jr., and R. H. Herman. Metmyoglobin reductase: Identification of a reduced nicotinamide adenine dinucleotide dependent enzyme from bovine heart which reduces metmyoglobin. Fed. Proc. 35: 1423 (Abstract 334), 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA 06 6374	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8a. DISSEM INSTR ^a	8b. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 13	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	61102A	3A161102B71R		02		065	
U. CONTRIBUTING							
C. CONTRIBUTING	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a (U) The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 005900 Environmental Biology; 012900 Physiology; 000350 Clinical Medicine, Food							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 07		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		76	
B. NUMBER: Not Applicable				FISCAL		3.1	
C. TYPE:				YEAR		0.8	
D. AMOUNT:				CURRENT		30	
E. KIND OF AWARD:				77		0	
F. CUM. AMT.						0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: Letterman Army Institute of Research Bioenergetics Division			
ADDRESS:				ADDRESS: Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Consolazio, C.F., DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5902			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hill, T.M. & Fults, R.D., DAC			
				NAME: Schnakenberg, D.D., MAJ, MS POC:DA			
22. KEY WORDS (Precede EACH with Security Classification Code) (U) Human Volunteers; (U) Caloric Needs of Soldiers; (U) Heat, Cold; (U) Nutrients and Body Composition; (U) Pulmonary Function; (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) Attainment and maintenance of optimum metabolism is of prime import to the Armed Forces and require a constant search for new methods for improving and evaluating physiological status. Status reflects nutriture and body hydration and may be affected by age, state of health, dietary constituents, vitamins, hormones, therapeutic agents, and varied stresses, all of which should be reflected in body composition changes and related physiological parameters. 24. (U) Energy metabolism will be studied with respect to respiratory, cardiovascular, hormonal and temperature regulation, and body composition changes. The biochemical and neurophysiological mechanisms underlying nutritional stress-induced changes in food and water consumption and their impact upon energy and fluid balances will be studied in appropriate animal models. The effects of dietary components (fat, protein, and CHO) will be evaluated singly or combined. 25. (U) 75 07 - 76 09. Studies evaluating the mechanisms by which stressors or nutritional-physiological status alter normal control of food and water consumption were continued. Studies in rats have shown that although polycythemia induced by transfusion of packed red blood cells will reduce hypoxic inhibition of food and water intake, polycythemia <u>per se</u> may play only a minor role in natural acclimatization to hypoxia. A small animal feeding and drinking data acquisition system has been developed and is currently being tested and evaluated. Two human studies were completed to evaluate protein requirements during heavy physical training. Two protein levels were investigated, the NRC allowance of 0.8 g/kg body wt., and the military allowance of 100 g protein/day at the 3600 and 3900 kcal expenditure level. The data are now being processed.							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A1061102B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	065	The Effect of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status

The following investigations have been conducted under this work unit:

- STUDY NO. 5 a. Effect of Induced Hypervolemic Polycythemia on the Feeding and Drinking Behaviors of Rats During Acute Exposure to 12% Oxygen
- STUDY NO. 6 Evaluation of Protein Requirements During Physical Training and Heavy Physical Activity

Study No. 5-a. Studies were conducted to determine the basic inhibitory mechanisms involved in hypoxic inhibition of appetite and thirst. Polycythemia induced in rats by either packed red blood cell transfusion or prolonged intermittent hypoxic exposure will reduce the severity of hypoxic hypophagia and hypodipsia. However, phlebotomy did not alter the enhanced food and water intake of hypoxia-induced polycythemic rats during the initial 24 hours of 12% oxygen exposure. Therefore, polycythemia per se may play only a minor role in the acclimatory response to hypoxia.

Study No. 6. Two human studies to evaluate the protein allowance of the Food and Agriculture Organization, the National Research Council-National Academy of Science, and of the military (AR 40-25) during heavy physical training were completed on the Metabolic Ward of LAIR. Information obtained included metabolic data for nitrogen and mineral balances, work performance, and body composition. The data are presently being processed and analyzed.

BODY OF REPORT

WORK UNIT NO. 065

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress

STUDY NO. 5-a

Effect of Induced Hypervolemic Polycythemia on the Feeding and Drinking Behaviors of Rats During Acute Exposure of 12% Oxygen

PROBLEM:

Consumption of inappropriate amounts of an otherwise nutritionally balanced diet will adversely affect nutritional status and may limit the capacity of the soldier to perform his assigned task. Voluntary food and water consumptions are transiently decreased in both man and animals during early acclimatization to high altitude. Certain metabolic disturbances, temporarily associated with mountain sickness syndrome, are primarily attributable to the hypophagic and hypodipsic effects of hypoxia. These disturbances include body weight loss, negative nitrogen and electrolyte balance, suppressed protein and lipid synthesis and possibly alterations in body fluid metabolism. Acute exposure of rats to hypoxic gas mixtures provides an effective model to study stress-induced modification of food and water intake. Furthermore, hypoxic activation of the body's appetite and thirst inhibitory mechanisms may contribute to a better understanding of the physiologic and biochemical factors involved in short-term control of food and water intake. The development of an automated system to monitor continuously (minute-by-minute basis) the food and water consumption of small animals will greatly enhance our capabilities to study the factors involved in the control of appetite and thirst.

RESULTS AND DISCUSSION OF THE RESULTS:

1. Studies to assess the role of polycythemia in the acclimatory response to hypoxia were conducted. Food intake during 14 hours of exposure to 12% O₂ was observed in rats rendered anemic (hematocrit = 25.7%) by phlebotomy, polycythemic (hematocrit = 56.9%) by intraperitoneal injection of packed red blood cells, and in saline-injected controls (hematocrit 41.7%). Polycythemic rats consumed greater ($P < .05$) amounts of diet (11.6 g) than either the control (5.4 g) or anemic (5.4 g) groups. In a second study, food consumption during an initial 24 hours of 12% O₂ exposure was compared in altitude-acclimatized polycythemic rats (21 days of 6 hrs 12% O₂, 18 hrs air; hematocrit = 53.3%); phlebotomized altitude-acclimatized normocythemic rats (hematocrit = 43.3%); control packed-red-blood-cell-injected polycythemic rats (hematocrit = 54.3%); and control normocythemic rats (hematocrit = 45.4%). Food intakes of the altitude-acclimatized polycythemic (16.4 g), altitude-acclimatized normocythemic (15.2 g), and control polycythemic

(12.1 g) groups did not differ but were greater ($P < 0.05$) than the control normocythemic group (7.3 g). The groups were re-exposed to 12% O_2 for 24 hours at 5, 10, and 15 days following the initial exposure. The hematocrits of the altitude polycythemic, altitude normocythemic, and control polycythemic groups normalized to that of the control normocythemic group. In contrast, the food intake of the control normocythemic group gradually increased to that of the other groups (14-16 g) during the repeated hypoxic exposures. An experiment with the feeding response of the rat being used to evaluate the temporal characteristics of adaptation, de-adaptation, and re-adaptation to an hypoxic environment was conducted. The data are currently being reduced and evaluated.

2. Considerable progress was made in the development of the Small Animal Feeding and Drinking Data Acquisition System. The hardware components of the system were received, assembled, tested, modified, and retested. Implementation of the system awaits the necessary software programming to process the raw data stored on magnetic tape.

CONCLUSIONS:

Although polycythemia induced by packed red blood cell transfusion prior to exposure will reduce the severity of hypoxic hypophagia, polycythemia per se may play only a minor role in the acclimatory response to hypoxia. This conclusion appears applicable to conditions of low to moderate oxygen demand, but may not apply to conditions of prolonged exhaustive physical activity.

RECOMMENDATIONS.

Continue evaluation and prepare reports on completed experiments. Develop software to test and implement the Small Animal Feeding and Drinking Data Acquisition System.

PUBLICATIONS:

1. Hannon, J.P., L.F. Krabill, T.A. Wooldridge and D.D. Schnakenberg. Effects of high altitude and hypophagia on mineral metabolism of rats. J. Nutr. 105: 278-287, 1975.
2. Schnakenberg, D.D., D.H. Sotak, and C.F. Consolazio. Food intake of polycythemic rats during hypoxic exposure. Fed. Proc. 35: 369, 1976 (Abstract).
3. Consolazio, C.F. and D.D. Schnakenberg. Nutrition and the responses to extreme environments. Fed. Proc. (in press).

PROBLEM:

The objective of the study was to evaluate the daily protein allowances recommended by the National Research Council-National Academy of Sciences, Food and Agriculture Organization, and the military (AR 40-25). These levels of protein intake will be studied at physical activity levels of 3000, 3300, 3600, and 3900 kilocalories per day. Protein is an expensive source of calories and it would be uneconomical to increase the protein intakes unless the additional allowances have beneficial effects on heavy physical training.

RESULTS AND DISCUSSION OF THE RESULTS:

Two human studies were completed on the Metabolic Ward of this Institute to evaluate the effects of protein allowances during heavy physical training. In Study I, two groups of five subjects each were placed on diets providing either 0.6 g or 1.43 g of protein per kilogram of body weight per day. During the initial 12 days of the study, the subjects had an energy expenditure of 3000 kilocalories per day. During the remaining 18 days on the study, the subjects had an energy expenditure of 3600 kilocalories per day. Study II was conducted with two groups of six subjects each, with the same protein intakes as employed in Study I. The study was conducted in the same manner as Study I, except that the energy expenditure during the 18-day phase was increased to 3900 kilocalories per day.

Information collected during the studies included measurement of changes in body composition, performance, and metabolism of nitrogen and minerals. The data are now being processed and analyzed.

CONCLUSIONS:

Reports will be prepared on the changes in nitrogen and mineral balances, performance, and body compartments.

RECOMMENDATIONS:

None until data have been statistically analyzed.

PUBLICATIONS:

1. Consolazio, C.F. Physical performance and some biochemical changes related to nutrition. Proceedings of the Xth International Congress of Nutrition, Kyoto, Japan, 3-9 Aug 75 (Abstracts, p. 21).

2. Consolazio, C.F., H.E. Sauberlich, H.L. Johnson, H.J. Krzywicki, T.A. Daws, and R.A. Nelson. Relationships of diet to the performance of the combat soldier. In: Nutritional Adaptations to the Environment. Publication in the International Biological Program. Coordinated by O.L. Kline and C.G. King, Human Adaptability Coordinating Office, University Park, PA, Feb 76, pp. 63-68.
3. Consolazio, C.F. Physical activity and performance of the adolescent. Chapter 11. In: Nutrient requirements in adolescence. Edited by John I. McKigney and H.N. Munro. Cambridge: MIT Press, 1976, pp. 203-221.
4. Fults, R.D. and C.F. Consolazio. Book Review: (on) Skinfolds of Youths 12-17 Years, United States. Am. J. Clin. Nutr. 28: 1079, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DAOA 6377	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DES'N INSTR ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF SUM
75 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	61102A	3M161102BS02				055	
B. CONTRACTOR	61102A	3A161102B71R				166	
C. CONTRACTOR	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Design of Military Biomedical Research Information Systems (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
00420 Computers; 009700 Mathematics and Statistics							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 07		CONT		DA		C-In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		76	
B. NUMBER: ^a Not applicable				FISCAL		7T	
C. TYPE:				CURRENT		77	
D. KIND OF AWARD:						3.0	
E. AMOUNT:						87	
F. CUM. AMT.						29	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a				ADDRESS: ^a Department of Information Sciences Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Chastain, R.V. JR., CPT, MS			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-5541			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
				ASSOCIATE INVESTIGATORS			
				NAME: Nelson, R.A., DAC			
				NAME: Langley, W.B., DAC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ^a							
(U) Digital Computers; (U) Data Base Management; (U) Data Files; (U) Biomedical Research Information; (U) Statistics							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To design, implement and document computer programs and program systems for the management of LAIR research data. Such programs and systems are essential in the mission area of LAIR in order to: (a) process the results of clinical and laboratory studies to derive their conclusions and to test applicability to specific military situations, (b) maintain and effectively utilize a repository of past research data for direct application to military problems and correlation with future approved military research (c) evaluate data reported in the open literature and available from non-military research to determine its applicability and to apply it to the military environment by correlation and transformation techniques, (d) evaluate proposals and research results in the contract research program for military applicability and determine if contract requirements have been met and analyses have been correctly performed, (e) support the requirements of the freedom of information act and other directives requiring access to data by providing timely access to information on past military research activities, (f) provide database and rapid analysis capability for information within the mission areas of LAIR in the event of mobilization.</p> <p>24. (U) General purpose computer programs will be used to the greatest extent possible. Where the unique information processing requirements of a specific research protocol cannot be met by available general purpose computer programs, special purpose programs will be developed.</p> <p>25. (U) 75 07 - 76 09. The principal extension to existing general purpose programs beyond routine maintenance of existing database management programs, has been in the area of graphic representation of data and reduced results. Special purpose programs were produced for military biomedical research projects such as microbiology, protein intake studies, neurophysiological and metabolic mechanisms, and dermatology.</p>							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Biomedical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.	166	Design of Military Biomedical Research Information Systems

The following investigations have been conducted under this work unit:

STUDY NO. 1	Data Processing Support to Biomedical Research (General Support)
STUDY NO. 2	Direct Computer Support to LAIR Departments

The major effort under Study No. 1 has been directed to the development of two graphics support systems. One system permits a graphics display on 35 mm microfilm. The other system provides graphic displays on graphic scope terminals as a means for real-time display of process control parameters. A new generalized scientific data base management system is being evaluated for potential installation and use by LAIR. Included in this report as a second study is a resume of the substantive research support provided the various departments of the Institute.

BODY OF REPORT

WORK UNIT NO. 166

Design of Military Biomedical
Research Information Systems

STUDY NO. 1

Data Processing Support to
Biomedical Research (General
Support)

PROBLEM:

The objective of this study is to provide software resources of a general nature. The resources to be provided include systems and programs that provide data processing capabilities considered useful to the military biomedical research conducted at LAIR.

RESULTS AND DISCUSSION OF RESULTS:

For general data processing support to LAIR investigators, several programs have been developed and are considered useful to processing data from a variety of applied experiments.

a. Department of Nutrition.

(1) Food Hygiene Division. A histogram plotting program has been developed to display graphically on 35 mm microfilm the frequency of occurrence of laboratory reportings with bacteria counts ranging over an established interval. Frequencies accumulated for a food class, item, and the organism common to all reportings are depicted; the user supplies plot titling, frequencies, and dependent variable scale values.

(2) Bioenergetics Division. A generalized graphics package available with the Terminal Control System supplied by the Lawrence Berkeley Laboratory (LBL) as part of the Treadmill Automated System (TAS) support library has been tested and used at LAIR. The system is being used to support graphical analysis on a Tektronix 4012 display terminal of energy expenditure data and is likewise supporting graphical analysis of data collected in the Alameda nutrition survey.

b. Department of Information Sciences.

Applications Division. A generalized data base management system, Scientific Information Retrieval (SIR), which supports management of hierarchical data bases with an interface capability to the statistical package for the Social Sciences (SPSS) and the University of California at Los Angeles Biomedical Statistical Package (BMDP), is being studied. The system is being tested at the University of Washington School of Public Health and Community Medicine, Department of Biostatistics. LAIR has inquired about its transportability to the CDC 7600/6600 computers at LBL and to the Data General C300 central minicomputer at LAIR.

CONCLUSION:

Greater returns on investments of programming resources are obtained through utilization of general purpose systems. After a certain programming requirement has been developed for a particular experimental process, usefulness to other experiments can be expanded.

RECOMMENDATIONS:

Studies into the feasibility of installing a local version of the SIR data management system should be continued.

STUDY NO. 2

Direct Computer Support to
LAIR Departments

PROBLEM:

Modern military biomedical research is being designed to incorporate recent technological advances in automatic data processing and data acquisition. The objective of this study is to provide the research departments of LAIR with information processing support required to conduct experiments and/or analyze data.

RESULTS AND DISCUSSION OF RESULTS:

Techniques are being analyzed that will effect a more responsive utilization of data base management through interactive and on-line processing systems. Studies continue in search for a generalized data base management system, addressable by investigators and easily interfaced with associated data management and analysis processes. The need for applied responsive systems exists as a straight-forward requirement. Among all data processing tasks, efforts are directed at making available to investigators any resources that will allow a more direct control and utilization of computer based equipment that performs data acquisition, reduction, and analysis tasks.

Recently, a central in-house minicomputer was procured which supports remote conversational processing. A significant commitment of support in systems analysis, design, programming and implementation of programmed processes is anticipated to aid investigators in their utilization of this sophisticated resource.

Computer support in FY76 and FY77 to departments at LAIR has increased substantially as personnel requirements within the Department of Information Sciences were satisfied and as investigative staffs became more familiar with data processing capabilities available. Remote user services of Lawrence Berkeley (LBL) offer a limited hands-on capability in a batch processing environment, and so, accomplishments

in this period do not reflect the development and utilization of responsive systems planned for in-house computer support.

Support given to nutrition studies is a collaborative effort in which the Department of Information Sciences is one of the participating departments. As such, the support provided to that area is described under Work Unit 086, Nutrition Studies in Support of DOD Food Program.

a. Department of Nutrition.

(1) Food Hygiene Division.

(a) All required reports of data contained in the 1974 and 1975 data bases have been completed. A summary of analysis of data collected during calendar year 1976 is being recorded on redesigned data input forms, use of which has shown a decrease in keypunch preparation time. An editor, which preprocesses data prior to permanent file entry, has been programmed and is presently being tested.

(b) A program has been developed and implemented which orders and formats a standard values file of unique data elements relevant to the microbiological data collection system. Creation of a standard values file, issued as a reference file by the editor, has made possible an improvement in the accuracy and completeness of the data base; exceptions are noted by the editor on the computer output.

(2) Bioenergetics Division.

(a) Using remote job entry to the CDC 7600-6600 computers at Lawrence Berkeley Laboratory (LBL), data processing support has been given to two long-term protein studies conducted by that division. Because of repeated hardware failure of the DYMEC data acquisition system and a requirement to support phase three of the protein study, conversion was required of existing energy expenditure data reduction and analysis programs from the LBL environment to the in-house MODCOMP II mini-computer. The program conversion effort, largely a rewrite of data acquisition and analysis programs, is in progress. Enhancements, such as signal error detection and real-time display of monitored data, are also being programmed. Additionally, acquisition of continuous oxygen uptake data, using a Digital Equipment Corporation PDM70 programmable data mover, has been achieved and is now an integral component of the data acquisition process.

(b) Development of a system was begun to support investigations on the neurophysiological and metabolic mechanisms controlling food and water consumption, and how these regulatory mechanisms are influenced by dietary components, environment and exercise. A data acquisition unit capable of continuously recording the feeding and drinking patterns of eight rats simultaneously was procured. Initial software development concentrated on reading the recorded data from a tape cassette attached to one of the communications

channels of the MODCOMP II minicomputer. No completely successful transfer of data has yet been accomplished. Further testing is required.

b. Department of Dermatology.

(1) Cutaneous Infection Division. Data processing support continues using the Dermatology Data Collection System (DDCS) designed and programmed in-house and remotely processed at LBL. Dermatology Clinic outpatient data acquired in March 1976 from a medical center and a field hospital, remains to be loaded to the permanent file before quarterly and yearly diagnosis frequency distribution reports can be generated. Data processing support using DDCS also continues for the purpose of managing and analyzing dermatology clinic outpatient data from two field hospitals defined as a separate study from the above described application.

(2) Cutaneous Protection Division. Data from 248 mosquito repellent effectiveness tests have been keypunched, scanned, and loaded to a data base managed by the Remote File Management System (RFMS). To support the research of investigators, qualified retrievals of data base components with reportings from applied basic statistical functions have been completed. Methods of more extensive statistical analysis to be applied to this data base are being studied. To achieve that analysis, linearization of the data base is required to create a file of coded records that can then be input to a statistical analysis system.

c. Department of Medicine.

(1) Metabolic Laboratory Division. Reduction and analysis of continuous oxygen uptake data acquired during two treadmill tests have been completed. Continued data processing support of follow-on testing is performed as required.

(2) A system required to manage and analyze patient medication intake and temperature response data is to be designed and programmed. Likewise, in-house hardware support for this application is also required.

d. Department of Biomedical Stress.

Military Performance Division. Data collected in muscle stress pre-testings, decoded from physiological tape recordings and printed on strip forms, were received for processing. Design of a keypunch data format and subsequent keypunching have been completed. Pre-test data have been established on file at LBL. Similarly, data from muscle stress post-testings, digitalized on the PDP-12 minicomputer located in the Nonionizing Radiation Division, have been unloaded from that system to paper tape and then transmitted to LBL for creation as a data file. Paper tape was required as a

medium for data storage because it is the only input/output medium common to the PDP-12 minicomputer and the large scale computers accessed at LBL. An editor to scan the source data files has been designed, programmed and is presently being tested. Methods of analysis of these data sets are being studied by the investigator.

d. Department of Comparative Medicine.

Animal Resources Division. A system to handle animal per diem billing charges was implemented at the beginning of FY76. Based on semiannual time-and-motion studies conducted by the Animal Resources Division (ARD), per diem rates are calculated for the twelve species of animals currently being maintained. Each month the total number of census days are added up and a total charge calculated using the per diem rates. This total charge is compared with the actual expenses incurred by ARD and the rates are then adjusted upward or downward until the charges equal the expenses. The computerized system uses coded census sheets and data files from the Decentralized Accounting Office System to perform these calculations and generate automatic billing charges to the various work units of each investigator using laboratory animals.

e. Program and Accounting Office.

(1) Minor modifications and enhancements were made to the Decentralized Accounting Office (DAO) System throughout the year. In some instances different breakouts of certain totals were provided; in others the data was presented in a different sequence. Nearly all programs in the DAO system underwent some changes to handle the transition in FY77.

(2) No progress on the Interfund Billing Subsystem was made. Several issues regarding the manipulation of the input data need to be resolved first. The system is designed to automate the manual procedures currently used to process the financial accounting transactions between LAIR and the Presidio.

f. Department of Administration.

Medical Audio/Visual Aid Division. An Audio/Visual Costing System was designed, programmed and tested and is ready to be implemented. This system bills each department and office FIC, via the DAO system, the costs of Audio/Visual work orders submitted by that department or office to the Medical Audio/Visual Aid Division, LAIR. A report which summarizes costs by FIC for each Department/Division is produced for use by management.

g. Department of Logistics. Development of a system to handle medical equipment calibration schedules for the Instrument Services Division was begun. This system will maintain an equipment master

file and produce listings of equipment due or overdue for calibration. Included for each unit of equipment is information such as nomenclature, model number, manufacturer, red tag number, and hand receipt holder.

PUBLICATIONS:

1. Fowler, J.L., D.L. Stutzman, J.F. Foster, W.H. Langley, and K.E. Trefz: Report of 1974 Microbiological Data Collection Program. Published as LAIR Report No. 28.
2. Fowler, J.L., D.L. Stutzman, J.F. Foster, W.H. Langley, and K.E. Trefz: Report of 1975 Microbiological Data Collection Program. Submitted to the Commander for clearance.
3. Fowler, J.L., D.L. Stutzman, J.T. Foster, and W.H. Langley: Selected Food Microbiological Data Collected Through a Computerized Program. LAIR Report.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUM'Y	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
	61102A	3A161102B71R	02	167			
XXXXXXXXXX							
XXXXXXXXXX CARDS 114(f)							
11. TITLE (Precede with Security Classification Code) ^a (U) Biochemical Factors Influencing Physiological Functioning (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
72 07		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER: Not Applicable				76		1	
c. TYPE:				FISCAL YEAR		.3	
d. AMOUNT:				CURRENT		8	
e. KIND OF AWARD:				77		0	
f. CUM. AMT.				0		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Herman, R. H., COL, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Human Volunteers; (U) Fever; (U) Temperature Regulation; (U) Adrenergic Mechanisms; (U) Adrenergic Blocking Agents; (U) Hormones; (U) Circadian Rhythm; (U) Lab Animals.							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23.(U) The soldier is subjected to stress, particularly in training and in combat. Heat stress causes ineffectiveness, disability and death in military personnel and merits attention especially because it should be completely preventable in training and usually in combat. The regulation of body temperature by appropriate therapeutic agents and other measures to prevent or minimize the adverse effects of heat production would materially enhance the effectiveness of basic training and combat operations by postponing fatigue, reducing the time required for heat adaptation and preventing heat injury.							
24.(U) Selected patients with thermoregulatory defects were investigated with regard to returning temperature regulation to normal and to determining the site of the defect in the regulating mechanism.							
25.(U) 75 06-76 09 One patient has been studied. This patient with SA hemoglobin developed heatstroke during basic training and was studied in 1972. Physiologic abnormalities detected in 1972 are unchanged at the present time. These abnormalities may have predisposed to or may be the result of the episode of heatstroke. This work unit is being terminated.							

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Bio-Medical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT	167	Biochemical Factors Influencing Physiological Functioning.

The following investigations have been conducted under this work unit:

STUDY NO. 2. Studies in patients with fever.

Heatstroke is a life threatening disease which affects military personnel engaged in training, field maneuvers, and combat operations, as well as during anesthesia and febrile illnesses. Heatstroke is closely related to environmental conditions, particularly to high ambient temperatures and humidity.

One patient with disordered temperature regulation was studied. This patient was previously evaluated following an episode of heatstroke. (See Annual Progress Report dated 30 June 1973). He developed heatstroke at the Air Force Academy, Colorado Springs, Colorado, during a physical proficiency test. He was a superbly conditioned athlete and neither the temperature nor the humidity were high enough to be considered predisposing factors. He is Black with hemoglobin SA phenotype, and he is the first of two such patients to develop heatstroke and to undergo evaluation. Reevaluation of this first patient revealed several abnormalities which were previously present and are unchanged. The abnormalities include an excessive rise in muscle temperature during exercise following the induction of systemic acidosis, an excessively high pulse rate for either the workload or the oxygen consumption, and a plateau in oxygen consumption which appears to be inappropriate for the workload or for the heart rate. It is not clear whether these abnormalities are the cause or the result of the heatstroke.

BODY OF REPORT

WORK UNIT NO. 167

Biochemical Factors Influencing
Physiological Functioning.

STUDY NO. 2

Studies in Patients with Fever

PROBLEM:

Heatstroke is a life-threatening disease which affects military personnel engaged in vigorous or strenuous physical activity (i.e., training, combat) particularly in association with high ambient temperature and humidity. The factors involved in the pathogenesis of heatstroke are not completely known but appear to be related to disturbances of body temperature regulation. A patient with apparent myoglobinuric renal failure, secondary to heatstroke which was induced by severe physical exertion, was studied.

RESULTS AND DISCUSSION OF THE RESULTS:

The first patient with myoglobinuria and renal failure was a 19-year-old Air Force Academy cadet with known sickle cell trait who became ill in April 1972. Early in April he had a mild upper respiratory infection which was not troublesome. On 7 April he played Rugby without any apparent difficulty. On 8 April following a two-mile run he developed hyperventilation, confusion, and syncope. When seen at the dispensary he was agitated, acutely ill, and complained of pain in his legs and muscle spasms were noted. No temperatures were recorded at this time. He was admitted to the Air Force Academy Hospital where his temperature was 101°F and there was evidence of renal, hepatic, and muscular involvement. The urine was bright red and positive for myoglobin. During hospitalization the SGOT, LDH, and CPK were markedly elevated. His temperature remained between 102 and 103°F. Hepatic dysfunction continued. There was progressive renal dysfunction presumably secondary to acute tubular necrosis. He was transferred to Fitzsimons General Hospital on 11 April because of the necessity of hemodialysis. With continued intensive treatment his renal, hepatic, and central nervous system functions returned to normal. Because of the variety of medical problems which he developed and the uncertainty of the diagnosis he was referred to the Metabolic ward for further evaluation. The particular train of events which this patient developed (central nervous system, liver, and kidney dysfunction and myoglobinuria) can best be explained by the diagnosis of heatstroke. This diagnosis is made less likely by several factors: 1) the patient is a well-trained and conditioned athlete, 2) the amount of exertion at the time of his illness was not unusual for him, 3) the environmental conditions were not stressful, i.e., low temperature and humidity. Nevertheless, heatstroke remains the best diagnostic possibility unless one postulates a rare and heretofore undescribed type of idiopathic myoglobinuria. He had

known sickle cell trait. It should be pointed out that there are reports of sudden death following exertion in individuals with sickle cell trait in the medical literature.

The second patient with secondary renal failure was a 17-year-old Air Force Academy cadet with known sickle cell trait who developed myoglobinuria and renal failure as a result of heatstroke following strenuous physical activity. His history, physical abnormalities, and clinical course are strikingly similar (if not virtually identical) to the course of the first cadet.

These two patients seem to represent a special form of myoglobinuric renal failure following heatstroke, since both were well trained, well-conditioned athletes whose illnesses developed in the absence of noteworthy environmental stress. The role of the altitude at the Air Force Academy (approximately 8,000 feet) as well as the influence of the sickle cell trait in the pathogenesis of their illness remains uncertain but neither are considered to be of importance in the clinical spectrum of heatstroke.

Both of these patients underwent extensive evaluation on the metabolic ward. Muscle temperature production was enhanced during exercise following the induction of systemic acidosis with ammonium chloride loading. Following ammonium chloride loading, muscle temperatures rose to between 0.5° and 1°C. higher than under identical control exercise conditions. The relationship of acidosis to the myoglobinuria in this condition and to the episodes of myoglobinuria in idiopathic myoglobinuria remain uncertain. It is possible that systemic acidosis may play a role in both conditions.

On reevaluation of the first of these two patients, the abnormalities which were previously detected were once again found. Exercise following the induction of systemic acidosis caused a greater rise in muscle temperature than did control exercise under identical conditions. In addition, the pulse rate rose to excessively high levels for the amount of work being performed. Oxygen consumption rose appropriately for a short time and then reached a plateau which was inappropriate for the workload and for the heart rate. These findings seem to point to abnormalities in cardiovascular, pulmonary, and muscular systems. Whether they are the cause of, or the result of, the previous heatstroke remains uncertain.

CONCLUSIONS:

Two Black Air Force Academy cadets both of whom had sickle cell trait developed myoglobinuric renal failure presumably secondary to heatstroke as the result of severe physical exertion. In both individuals an enhanced muscle temperature response during exercise resulted from systemic acidosis induced by ammonium chloride loading. Whether

their illnesses represent a unique biochemical defect which is limited to the muscle membrane, or whether the sickle cell trait participates in some as yet unknown manner remains uncertain.

RECOMMENDATIONS:

The studies in these two patients have been important in demonstrating a defect which is both reproducible and measurable. These studies should be extended by determining the effect of ammonium chloride induced acidosis on muscle temperature during exercise in volunteer subjects, especially in Black subjects who have sickle cell trait (SA) and sickle cell disease (AA hemoglobin phenotype). Until the appropriate control studies are performed, the significance of the studies in these two patients must remain uncertain. This work unit is being terminated.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTN ^a	9a. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102B71R	02	168			
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(F)						
11. TITLE (Precede with security Classification Code) ^a							
(U) The Effects of Diet Upon Respiration Metabolism							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
012900 Physiology; 006500 Food							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 02		76 09		DA		C. In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				b. PRECEDING		c. FUNDS (In thousands)	
b. NUMBER: Not Applicable				76		1.6	
c. TYPE:				FISCAL		0.4	
d. AMOUNT:				77		5	
e. KIND OF AWARD:				77		0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: Letterman Army Institute of Research Bioenergetics Division			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Consolazio, C.F., DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5092			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Dramise, J.G., DAC			
				NAME: Fults, R.D., DAC POC:DA			
22. KEYWORDS (Precede EACH with security Classification Code)							
(U) Human Volunteers; (U) Respiratory Function; (U) Diffusing Capacity, (U) Lung Compartments; (U) Nutritional Stress; (U) Dietary Modifications							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.)							
<p>23. (U) It is important that respiratory efficiency be enhanced, if possible, to enable the soldier to respond more adequately to metabolic stress. The respiratory system may be altered with various acute and chronic changes in diet and environmental conditions encountered by military personnel. Studies will be initiated to measure the effect of nutritional and environmental manipulation upon (a) the respiratory system; (b) acid/base relationships; (c) blood gases; (d) cardiovascular responses, and (e) hemodynamic factors. The derived data may permit the development of recommendations to improve the respiratory efficiency of the soldier.</p> <p>24. (U) Studies will evaluate (a) the gaseous nitrogen exchange in humans during steady state conditions and with various protein intake levels; (b) the effects of varied levels of nutrient intake (protein, fat, carbohydrate) as they relate to changes in pulmonary function - primarily pulmonary diffusion capacity during rest and activity; and (c) hemodynamic responses resulting from dietary influence.</p> <p>25. (U) 75 07 - 76 09. A study on the effects of a high fat meal on pulmonary function indicates that elevated exogenous triglyceride levels (above 150 mg/100 ml) do not hinder gas diffusion, either at low or high altitude (4300 m), even with the addition of physical stress. The results of a second study indicate that a physiological adjustment period of as long as three days may be required for individuals returning to a low elevation (1600 m) following a sojourn at high altitude (4300 m).</p>							

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	168	The Effects of Diet Upon Respiration Metabolism

The following investigations have been conducted under this work unit:

- STUDY NO. 1 The Effects of a glucose Meal on Human Pulmonary Function at 1600 and 4300 m Altitude
- STUDY NO. 2 Effects of a High Fat Meal on Pulmonary Function at 1600 and 4300 m Elevations
- STUDY NO. 3 The Effects of a High Protein Meal on Pulmonary Function at 1600 and 4300 m Elevations
- STUDY NO. 5 Changes in Pulmonary Volumes With Relocation to 1600 m Following Acute Translocation to 4300 m

Study No. 1. Results of this study have been published (Aviat. Space Env. Med. 46: 365, 1975).

Study No. 2. See Annual Progress Report for FY 75, page 135.

Study No. 3 Studies using high protein meals at both 1600 m and 4300 m are being processed for statistical analyses. However, a preliminary review of the data suggests no significant changes occurred in pulmonary function as a result of the ingestion of a high protein meal.

Study No. 5. The results of the investigation indicate that reacclimation to a low altitude (1600 m) following a sojourn at a high altitude (4300 m) may require an interval of as long as 3 days for adjustment of certain body functions.

BODY OF REPORT

WORK UNIT NO. 168

The Effects of Diet Upon Respiration
Metabolism

STUDY NO. 1

The Effects of a Glucose Meal on Human
Pulmonary Function at 1600 m and 4300
m Altitude

PROBLEM:

Respiratory efficiency should be enhanced, if possible, to enable the soldier to respond more adequately to physical and environmental stress. The respiratory system may be altered with various acute and chronic changes in diet and environmental conditions encountered by military personnel. The objective of a series of studies was to measure the effect of nutritional and environmental manipulation upon (a) the respiratory system, (b) acid/base relationships, (c) blood gases, (d) cardiovascular responses, and (e) hemodynamic factors. The derived data from the investigations may permit the development of recommendations to improve the respiratory efficiency and performance of the soldier.

Specific studies were designed to evaluate (a) the gaseous nitrogen exchange in humans during steady state conditions and with various protein levels, (b) the effects of varied levels of nutrient intake (protein, fat, and carbohydrate) as they relate to changes in pulmonary function, primarily pulmonary diffusion capacity during rest and exercise, and (c) the hemodynamic responses resulting from dietary influence and pulmonary-respiratory efficiency.

RESULTS AND DISCUSSION OF THE RESULTS:

The data obtained following glucose ingestion indicate that pulmonary gas diffusion is increased. The elevated pulmonary diffusion capacity suggests an increase in gas exchange at the alveolar-capillary level following the ingestion of a glucose meal for individuals transported to high altitude.

CONCLUSIONS:

The conclusions of this study have been published (Aviat. Space Environ. Med. 46: 365, 1975).

RECOMMENDATIONS:

The next study should involve the use of various carbohydrates to evaluate the most efficient source.

PUBLICATIONS:

J.G. Dramise, C.M. Inouye, B.M. Christensen, R.T. Fults, J.F. Canham and C.F. Consolazio. The effects of a glucose meal on human pulmonary function at 1600 m and 4300 m altitude. Aviat. Space Environ. Med. 46: 365, 1975.

STUDY NO. 3

The Effect of a High Protein Meal on
Pulmonary Function at 1600 and 4300 m
Elevations

PROBLEM:

The military has a great interest in maintaining the maximal efficiency of troops. Any means of dietary manipulation which would enhance the work performance would be acceptable for use in troops in combat or maneuvers.

RESULTS AND DISCUSSION OF THE RESULTS:

Data obtained from this study in which protein meals were fed to adult male subjects at both 1600 and 4300 m are still being processed for statistical analyses. However, a preliminary survey of the data suggests no significant changes occurred in pulmonary function as a result of the ingestion of a high protein meal.

CONCLUSIONS:

None.

RECOMMENDATIONS:

None.

PUBLICATIONS:

Rodkey, W.G., J. G. Dramise, J.P. Hannon, and T.J. Bucci. The use of arterialized ear blood to assess the acid base and blood gas status of dogs. Fed. Proc., 35: 839, 1976 (Abstract).

STUDY NO. 5

Changes in Pulmonary Volumes With Relocation to 1600 m Following Acute Translocation to 4300 m

PROBLEM

The physical performance of military personnel is of prime concern to the military. Since a large portion of the basic and airborne training programs are devoted to increasing physical fitness, an increase in respiratory function of the individual would be very beneficial to military planning.

RESULTS AND DISCUSSION OF THE RESULTS:

In studies conducted on 12 volunteer subjects, vital capacity (VC) and expiratory reserve volume (ERV) significantly decreased during a 3-day sojourn at 4300 m, while pulmonary ventilation (\dot{V}_E), tidal volume (\dot{V}_T), total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV) were elevated. Acid-base parameters showed typical changes associated with translocation to high altitude. Thus, the partial pressure of oxygen in arterial blood (P_{aO_2}) and the oxygen saturation of hemoglobin in arterial blood (SaO_2) were immediately reduced upon translocation to 4300 m, while the compensatory reduction in arterial HCO_3^- concentration was delayed temporarily by 24 hours; pH, however, remained essentially unchanged throughout the sojourn. Upon relocation to 1600 m, there was a gradual return of VC, ERV, TLC and RV to prealtitude values. The functional residual capacity of \dot{V}_E and \dot{V}_T remained elevated through the third day of relocation to 1600 m. The partial pressure of carbon dioxide in the arterial blood (P_{aCO_2}) and the arterial HCO_3^- concentration showed a slight delay in returning to prealtitude values upon relocation, while the remainder of the acid-base measurements returned to prealtitude values within 24 hours.

CONCLUSIONS:

The results of this investigation indicate that a physiological adjustment period as long as 3 days may be required for individuals returning to low or moderate elevations (1600 m) from a 72-hour sojourn to high altitude (4300 m).

RECOMMENDATIONS:

This series of studies on the interactions of diet and altitude has been concluded.

PUBLICATIONS:

Dramise, J.G., C.F. Consolazio, and H.L. Johnson. Changes in pulmonary volumes with relocation to 1,600 m following acute translocation to 4,300 m. *Aviat. Space Environ. Med.* 47 (3): 261-264, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY 76 04 02	4. KIND OF SUMMARY H. TERMINATION	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8. DISSEM INSTRN NL	9a. SPECIFIC DATA- CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3A161102B71R	02	169			
b. XXXXXXXXXX	61102A	3A061102B71R	02				
c. XXXXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code)* (U) Comparative Pathology of Animals Maintained and Utilized in Biomedical Research							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 002600 Biology							
13. START DATE 72 07		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C In-House	
17. CONTRACT/GRANT a. DATES EFFECTIVE: b. NUMBER* Not Applicable c. TYPE: d. KIND OF AWARD:				18. RESOURCES ESTIMATE a. PRECEDING 76 FISCAL YEAR 7T CURRENCY 77 b. PROFESSIONAL MAN YRS 8.0 2.0 0 c. FUNDS (in thousands) 160 30 0			
19. RESPONSIBLE DOD ORGANIZATION NAME* Letterman Army Institute of Research ADDRESS* Presidio of San Francisco, CA 94129 RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: (415) 561-3600				20. PERFORMING ORGANIZATION NAME* Letterman Army Institute of Research ADDRESS* Pathology & Comparative Studies Div Department of Comparative Medicine Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME* Mellick, P.W., MAJ, VC TELEPHONE: (415) 561-3855 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: Bucci, T.J., LTC, VC NAME: POC:DA			
21. GENERAL USE Foreign Intelligence Not Applicable							
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Comparative Pathology; (U) Laboratory Animals; (U) Human Analogues; (U) Military Research Support							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) Biomedical research depends upon the proper use of healthy laboratory animals. Broad objectives of this work unit are: 1) to characterize diseases or unique characteristics of laboratory animals, for study as animal models of conditions affecting humans, and 2) to provide complete pathology service for studies involving animals. 24. (U) Three spontaneous disease conditions affecting laboratory animals were characterized, and a model for respiratory tract injury was developed. Complete pathology services, including electron microscopy, were provided. 25. (U) 76 04-76 09. Spontaneous mineralization of myocardium of DBA mice was determined tentatively to originate in mitochondria. Canine distemper was discarded as an appropriate model for development of immunohistochemical methods for rapid diagnosis of viral diseases. Study of three dogs having panosteitis led to rejection of the hypothesis that it is a communicable infectious disease. A model was developed in rats in which to study occupationally-related pulmonary injury. Pathology services comprised 1316 cases including 9183 paraffin blocks, 28,461 slides, 1253 CBCs, 821 SMA 6/12 determinations, 103 urinalyses, 478 tests for parasites, 659 microbiologic cultures including 85 for virus isolation, and 5693 micrographs were prepared. Specific disease problems encountered included an outbreak of upper respiratory infection in 14 newly acquired cats. Thirteen of these animals were clinically ill. Five animals died. Viruses isolated from these animals included feline herpes virus, feline syncytial virus and feline calicivirus.							

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Biomedical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.	169	Comparative Pathology of Animals Maintained and Util- ized in Biomedical Research

The following investigations were conducted under this work unit:

STUDY #4 Feasibility of the Use of Immunoenzyme-Labeled
Antibody in the Histopathological Diagnosis of
Viral Laboratory Animal Diseases

A technique to detect directly the presence of viral antigen is being modified for use in both fresh frozen and fixed tissues. When used on fresh tissue, the technique will provide information in addition to that obtained from virus isolation. In the case of fixed tissue when virus isolation is impossible, this technique, with appropriate modification, may provide the only method for detecting and identifying the causative agent of the disease. One model has been tested and discarded because of technical difficulties. A second model system designed to avoid these difficulties is being evaluated.

STUDY #5 Thyroid and Adrenal Function in Mexican Hairless
Dogs

The high incidence of skin disease and the clinical appearance of the LAIR colony of Hairless dogs are highly suggestive of an underlying thyroid or adrenal disorder. Knowledge of their endocrine status is necessary to define completely their usefulness as models of skin disease of soldiers. Serum specimens are being collected for assay.

STUDY #6 Myocardial Mineralization in Selected Strains of
Mice

To study the sequence of biochemical and morphological events that occur in spontaneous myocardial mineralization of DBA/2 mice, samples of serum and myocardium were collected from an initial group of 112 animals representing different age groups. Serum electrophoresis, lactic dehydrogenase and creatine phosphokinase values varied so greatly that it was not possible to identify affected animals. Preliminary morphologic studies, using both light and electron microscopy, revealed the mineralization to be in mitochondria and myofibrils. Additional tissues have been collected from 149 mice for light microscopic and ultrastructural examination in an effort to determine the primary locus of mineralization.

STUDY #8

Epithelial Regeneration in the Upper Respiratory
Tract Following Acute Oxidant Injury

To study the cytodynamics of upper airway epithelial regeneration following acute chemical injury, weanling rats were exposed to a sublethal concentration (0.8 ppm) of ozone for 8 hours. These animals and a group of unexposed controls were then given an intraperitoneal injection of 500 μ C of 3 H thymidine. Animals were killed at intervals after the isotope injection. Respiratory tissues were collected and preserved for light microscopy, transmission electron microscopy, scanning electron microscopy and autoradiography. Preliminary histologic and scanning electron microscopic examination of tissues from ozone-exposed rats indicates that epithelial damage sufficient to stimulate repair processes was produced. The duration of cell cycle phases will be determined by using light microscopic autoradiography. Epithelial differentiation will be documented by electron microscopic autoradiography. The techniques used in this study will be standardized so that they may be used as a model system in subsequent studies to assess the effects of occupationally related pulmonary injury in military personnel.

BODY OF REPORT

WORK UNIT NO. 169

Comparative Pathology of
Animals Maintained and
Utilized in Biomedical
Research

STUDY #4

Feasibility of the Use of Immunoenzyme-Labeled
Antibody in the Histopathological Diagnosis of
Viral Laboratory Animal Diseases

PROBLEM:

Healthy laboratory animals are a vital resource for Army medical research. Diseased animals are wasteful of this resource and the loss is compounded when experiments cannot be evaluated properly because of intercurrent disease in the research animals. Accurate and expeditious means to detect and diagnose diseases, particularly viral diseases are therefore highly desirable. It has been impractical and time-consuming to use standard procedures to culture, isolate, and identify the virus(es) that cause disease in laboratory animals. Techniques using enzyme-labeled specific antibody have been used for the rapid detection and identification of viral antigens in human tissue. The advantage of these techniques lies in their rapidity, sensitivity, and reliability. This method will be adapted to detect and identify viral antigens in fixed and frozen tissue as a rapid and economical means for diagnosis of specific viral diseases of laboratory animals. When the technique has become a useful standardized tool for one model system, it will be adapted by using appropriate antisera for quick identification of other specific viral antigens which may be present in the tissues of both sick and apparently healthy laboratory animals.

RESULTS AND DISCUSSION OF THE RESULTS:

Techniques which detect antigen in tissue by using specific enzyme-labeled antibody are being adapted for routine diagnosis of viral diseases in laboratory animals. Trivalent hyperimmune canine serum prepared against canine distemper virus, canine adenovirus I, and Leptospira canicola was obtained from a commercial source. This serum was conjugated with horseradish peroxidase. Frozen tissue and tissue fixed in formalin were obtained from animals in which canine distemper had been diagnosed by histopathologic examination. These tissues were tested with the labeled antiserum. Non-specific staining made this test system unsuccessful. Lack of specificity may be attributed to preparation of the serum against multiple antigens or preparation in the same species as the tissue being tested.

Because of these problems, feline herpes virus was chosen as an alternate model system. This isolate was obtained from a naturally occurring

outbreak of upper respiratory disease in cats at LAIR. Hyperimmune serum to this feline herpes virus isolate was prepared in rabbits and is currently being evaluated.

CONCLUSIONS:

Commercially available trivalent hyperimmune canine serum is not a suitable source from which to prepare enzyme-labeled antibody for diagnostic purposes; feline herpes virus with locally produced rabbit antiserum is a more promising system.

RECOMMENDATIONS:

A model system must be found to develop methodology for peroxidase-labeled antibody diagnosis of viral diseases. Antisera to numerous laboratory animal diseases should be evaluated.

PUBLICATIONS:

None.

STUDY #5

Thyroid and Adrenal Function in Mexican Hairless Dogs

PROBLEM:

The Mexican Hairless dog may be a valuable model for study of conditions affecting soldiers' skin. The clinical appearance of the dogs suggests they may have thyroid or adrenal insufficiency. Assessment of their endocrine status is fundamental in evaluating their usefulness as a model. Serum has been collected periodically and stored for specific radioimmunoassay.

RESULTS AND DISCUSSION OF THE RESULTS:

Samples have been obtained and stored at -70°C . Results are not available because personnel were not available to adapt assay procedures to canine blood.

CONCLUSIONS:

None.

RECOMMENDATIONS:

The stored samples should be held, and others obtained; the assay should be developed and performed under a work unit which supports study of these animals. The present study will be terminated.

PROBLEM:

DBA/2 mice have a low incidence of mammary tumor and are therefore used in large numbers for studies of long duration. However, a high percentage of mice of the DBA/2 strain develop massive myocardial calcification if allowed to reach old age. Even though mice are severely affected, they appear to be clinically normal. This study is designed to define more precisely the morphologic and biochemical progression of this disease, to discover if possible the initial locus of mineralization, and to determine the limits this disease places on the utility of the DBA/2 mouse strain for other research.

RESULTS AND DISCUSSION OF THE RESULTS:

A breeding colony of DBA/2 mice was established to provide animals for this study. Serum and tissues were collected from 112 mice. This sample represented various age groups of DBA and control animals. Hearts from selected animals were processed for light and electron microscopy and the remaining tissues were stored. Serum electrophoresis, lactic dehydrogenase and creatine phosphokinase determinations were done on individual and pooled serum samples. Results of the enzyme analyses and serum electrophoresis varied so greatly that it was not possible to evaluate the data. The extreme variability probably resulted from improper calibration of newly acquired automated equipment for analyzing components of mouse serum. The preliminary light and electron microscopic findings showed mineral deposits to be in myofibrils and mitochondria. Hearts were collected from an additional 149 mice of different ages. These tissues are being processed for light and electron microscopic studies to determine the primary locus of myocardial mineralization.

CONCLUSIONS:

None.

RECOMMENDATIONS:

Following completion of the morphologic evaluation, this study should be terminated.

PUBLICATIONS:

None.

PROBLEM:

Upper respiratory infection is the most common illness that affects military personnel in both training and in combat situations. Soldiers are frequently exposed occupationally to airborne chemical agents that damage respiratory tissues. Epidemiological evidence and clinical experience indicate that chemically induced respiratory injury can predispose to upper respiratory infection. Knowledge of basic mechanisms by which upper respiratory epithelium is repaired is inadequate and is required so that treatment and clinical management of patients with upper respiratory injury can be improved. The objective of this study is to develop a laboratory animal model for the study of basic repair mechanisms in the respiratory tract.

RESULTS AND DISCUSSION OF THE RESULTS:

Upper respiratory epithelial injury was produced in rats by subjecting them to non-lethal concentrations of a highly corrosive gas (ozone). Exposed and control animals were given 500 μ Ci of ^3H thymidine by intraperitoneal injection immediately following the exposure period. Exposed and control animals were killed at intervals following injection of the isotope. Trachea and lungs of each animal were preserved for examination by light microscopy (LM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and autoradiography. Initial LM and SEM examination of exposed animals revealed that the degree of epithelial damage should be sufficient to stimulate repair processes. Initial light microscopic autoradiographic preparations indicate that they are of sufficient quality and sensitivity to study the cytodynamics of upper airway epithelial regeneration. With these techniques, the duration of cell cycle phases in ozone-exposed and control animals will be determined at two levels of the respiratory tract. Epithelial proliferation and differentiation will be studied further by using electron microscopic autoradiography. The results of this study will provide insight into the basic mechanisms involved in respiratory epithelial regeneration following injury. The standardized techniques developed in this study will be applicable to future investigations of repair mechanisms in the upper respiratory tract.

CONCLUSIONS:

Exposure of rats to 0.8 ppm ozone for eight hours produces sufficient damage to stimulate epithelial regeneration and provides a valuable model for the study of upper airway response to injury.

RECOMMENDATIONS:

Because of the change in research funding structure to Single Program Element Funding (SPEF) in FY 77, this study should be continued under 3M16162BS02, Basic Mechanisms of Recovery from Injury.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6102	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3M161102BS02				065	
b. CONTRIBUTING	61102A	3A161102B71R		03		126	
c. CONTRIBUTING CARDS 114(f)							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Behavioral Effects of Stress on the Mechanisms of Recovery from Injury							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
005900 Environmental Biology; 013400 Psychology; 016200 Stress Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 08		Cont		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER: Not Applicable				76		3.5	
c. TYPE:				FISCAL		7T	
d. AMOUNT:				CURRENT		1.9	
e. KIND OF AWARD:				77		7.5	
f. CUM. AMT.						115	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Military Stress Division			
				Department of Biomedical Stress			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Leibrecht, B. C., CPT, MS			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-2905			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Lloyd, A. J., MAJ, MS			
				NAME: O'Mara, P. A., CPT, MS			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Performance; (U) Primate Bloat							
(U) Leishmaniasis; (U) Stress and Disease; (U) Psychological Stress							
23. TECHNICAL OBJECTIVE. ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The severe stress encountered in warfare may influence the soldier's susceptibility to diseases and impair his ability to perform combat essential activities with maximum efficiency. The objectives of this research are to investigate in the laboratory the effects of emotional stress upon the development of selected disease processes and response patterns. Research is also conducted under field conditions to examine the effects of experimental stress upon the abilities of soldiers to perform various military activities.</p> <p>24. (U) Animals or human subjects are subjected to conditions which produce stress of varying intensity and duration. The effects of stressors are confirmed biochemically and through observation of changes in behavioral and physiological indices. For laboratory studies with animals, experimental stress is then related to the susceptibility and severity of disease processes. Human experimentation has been restricted to the evaluation of the effects of simulated combat operations upon performance.</p> <p>25. (U) 75 08 - 76 09 Laboratory investigations have shown that guinea pigs exposed to stress are more seriously infected by <u>Leishmania enriettii</u> parasites. Studies of acute bloat in subhuman primates have begun. Data concerning behavioral and biochemical characteristics of individual animals are being obtained. These data will be related to the occurrence of bloat in individual animals in subsequent studies. Recently completed studies have demonstrated the usefulness of recording physiological data under field conditions. Physiological data were found to be sensitive indicators of the individual soldier's responses to simulated combat conditions and were found to be related to performance abilities under these conditions.</p>							

^aAvailable to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161102B71R	Research in Biomedical Sciences
TASK NO.	03	Psychiatry
WORK UNIT NO.	126	Behavioral Assessment of Stress in Health and Disease Processes

The following investigations have been conducted under this Work Unit:

STUDY NO. 1 Stress and Cutaneous Leishmaniasis

STUDY NO. 2 Primate Acute Gastric Dilatation

STUDY NO. 3 SUPEX - CDEC, Fort Ord

STUDY NO. 4 Project 60 - West Point

Study No. 1. Nineteen guinea pigs were inoculated with Leishmania enriettii organisms; the course of the infection was monitored for several weeks. One group of animals was exposed one hour each day to a warning stimulus which was paired with brief electric shock; and a second group was chronically stressed by means of physical restraint for 6 hours each day. Both stress conditions produced ulceration earlier than it was noted in the third group of "un-stressed" animals. The resulting lesions also were more extensive and longer lasting in the stressed animals. Available data suggest that various stressors encountered in the military environment may play an important role in altering individual susceptibility to disease, recovery from disease, and may be determinants in primary and secondary effects of trauma.

Study No. 2. Rhesus monkeys have been successively adapted to the experimental rooms and restraint chairs in preparation for the imposition of stress episodes. Behavioral testing involving the presentation of novel objects has been completed. Videotape recordings from these tests and from earlier colony samples have been used in developing a behavioral taxonomy. Preliminary baseline measures of gastrointestinal emptying times and urinary 17-OHCS levels have been obtained.

Study No. 3. Heart rate was recorded in players performing a periscope tracking task while receiving direct or indirect weapons fire in a fortified bunker. Subjective ratings of anxiety and stress were also obtained. The results showed heart rate to be a sensitive index; it reflects both anticipatory and responsive components and successfully differentiates variables, such as the weapon. Selected heart rate parameters also correlated well with tracking performance scores.

Study No. 4. Surface electromyographic recordings were obtained from females during isometric and isokinetic tests. Tape recorded data have been digitized and converted to computer storage in preparation for computer analysis.

BODY OF REPORT

WORK UNIT NO.	126	Behavioral Assessment of Stress in Health and Disease Processes
STUDY NO.	1	Stress and Cutaneous Leishmaniasis

PROBLEM:

Infectious diseases in troops under field and garrison conditions represent a significant cost to the military in terms of lost manhours and in the cost of medical support required for treatment. Available data suggest that various stressors encountered in the military environment may play an important role in altering individual susceptibility to disease, recovery from disease, and may be determinants in primary and secondary effects of trauma. The present investigation was initiated to explore stress-disease relationships by using an animal parasitic infection which poses potential hazards to troops operating in tropical and sub-tropical environments. The disease was also selected for evaluation as a possible model for future stress-disease studies.

RESULTS AND DISCUSSION OF THE RESULTS:

Nineteen adult guinea pigs were divided into three experimental groups and inoculated with 5×10^5 Leishmania enriettii amastigotes. One group of six animals and another group of seven animals were then chronically stressed for five weeks. Animals in the first stress group were placed in boxes for one hour each day where they were periodically exposed to a tone stimulus which was randomly paired with brief electric shock. The second stress group was physically restrained for six hours each day. The third group of six animals was not experimentally stressed. During the first several days after treatment, animals were examined daily for the presence of lesions, nodules, or tissue necrosis. Weekly examinations were conducted after infection was apparent. Under the conditions stressed animals in the present experiment developed lesions earlier than control animals. Several weeks following infection the lesions of stressed animals were typically worse than those of control animals, and a longer time was required for healing.

CONCLUSIONS:

Stress produces marked effects in guinea pigs inoculated with Leishmania enriettii organisms. The cutaneous leishmaniasis develops sooner, lasts longer, and the lesions are worse in stressed than in unstressed guinea pigs. Data based on the gross pathology are not sufficiently analytical to be considered a precise method for quantifying lesion severity. The stress-disease model is valuable and should be used when valid and reliable methods of recording data are resolved.

RECOMMENDATIONS:

Further experiments on this study should be suspended until appropriate analytic techniques are established. Experimental design should be revised and expanded so that it includes biochemical testing and other analytic techniques which can accurately quantify the severity of the lesions. The same stress-disease model should be used again.

PUBLICATIONS: None.

Study No. 2. Primate Acute Gastric Dilatation

PROBLEM:

Spontaneous acute gastric dilatation (bloat) in primate is a serious problem in the research laboratory because it causes death of valuable primates, disrupts experiments, requires intensive veterinary support, and consumes monetary resources. What little is known about the cause of the syndrome suggests that a combination of stressful conditions and/or feeding procedures may play a major etiologic role. Individual organismic factors have also been implicated. A major purpose of this study is to determine the behavioral, psychophysiological, and stress factors which may play a role in primate bloat. The results of this approach will not only contribute to the conservation of valuable primate resources within the military laboratory, but also will serve in the development of a laboratory setting for initial studies in the reduction and control of inappropriate stress responses.

Progress in studying the bloat syndrome has been hampered by the lack of a valid experimental paradigm. Accordingly, the second major objective is to establish a non-surgical technique for reliably producing bloat in rhesus monkeys.

RESULTS AND DISCUSSION OF THE RESULTS:

Baseline videotaping in the colony has been completed for the first half of the subjects. Behavioral tests involving the presentation of novel objects to each monkey have been completed and videotaped. Based on the videotape recordings from both of these phases, a working behavioral taxonomy has been developed for use in constructing behavioral profiles. Preliminary baseline measures of gastrointestinal emptying times have been obtained using a dye technique. Preliminary baseline measures of 17-hydroxycorticosteroid levels in 24-hour urine samples have been determined.

CONCLUSIONS:

The behavioral testing procedures adapted for use in studying individual differences appear to be working well. These procedures yielded consistent differences between animals in their manner of responding to novelty,

differences which generally matched casual observations. Extreme stereotypy and restriction of behavior in the unstructured colony situation limited the use of colony observations for behavioral profiling purposes. The dye technique for measuring gastrointestinal emptying times, as modified for use in the present study, appears to be adequate.

RECOMMENDATIONS:

Observations to be used for behavioral profiling are best made in a structured test setting, as opposed to the undisturbed colony setting. Upon completion of profile validation, the development of a controlled bloat response using non-surgical procedures should be continued.

PUBLICATIONS None.

Study No. 3. SUPEX-CDEC, Fort Ord

PROBLEM:

The relationship between stress and performance is a complex one, especially under field conditions. Data from the laboratory cannot necessarily be applied to "real life" situations because of the difficulty in reproducing realistically threatening stressors in the laboratory. This makes it especially important to study stress and its potentially degrading influence on performance under realistic field conditions.

Psychophysiological indices offer distinct advantages over the more standard biochemical indices of stress. The former are non-invasive, continually accessible, and immediately responsive to discrete events. Thus, psychophysiological indices have much to offer the field study of stress.

A field study ("SUPEX" - Suppression Exercise) planned by the Combat Development Experimentation Command (CDEC) offered an opportunity to study stress under realistic conditions. The primary objectives were: (a) to field test newly acquired equipment; (b) to determine the sensitivity of heart rate to a field stressor as it interacted with performance task demands; (c) to evaluate possible relationships between heart rate and stress-induced changes in performance.

RESULTS AND DISCUSSION OF THE RESULTS:

Heart rate was recorded in CDEC players performing a tracking task while receiving fire from direct-fire weapons (M-60, M-139, MK-19) and indirect-fire weapons (60 mm and 81 mm mortars, 105 mm and 155 mm howitzers). Players were stationed in a fortified protective bunker while attempting to maintain visual contact through a periscope with a moving target up-range. Subjective ratings of anxiety and stress were also obtained before and after a series of trials.

Heart rate patterns typically showed acceleration before and during each trial, with gradual deceleration following the end of the trial. Heart rate acceleration was greater for faster rates of fire (relevant to direct-fire weapons only) and larger weapons than for the slower rates and small weapons. Selected heart rate parameters correlated significantly with tracking performance scores. Subjective ratings of stress and anxiety did not correlate significantly with heart rate parameters or performance scores.

CONCLUSIONS:

Heart rate is a sensitive index. It reflects both anticipatory and responsive components, and successfully differentiates the weapon and rate of fire. Whether or not the observed heart rate patterns represent stress responses, arousal, or some other dimension is not clear. Heart rate parameters offer potential for predicting responses to stress and performance under stressful conditions. Psychophysiological correlates of performance may depend on specific task conditions.

RECOMMENDATIONS:

Further research is needed to test other psychophysiological measures in field studies of stress and to evaluate the relationships between psychophysiological and biochemical indicators of stress.

PUBLICATIONS: None.

Study No. 4 Project 60 - West Point

PROBLEM:

Recent testing at United States Army Military Academy (USAMA), West Point, before the arrival of the first female plebes demonstrated that the best female performance score on the Physical Aptitude Examination (PAE) test was below that of the average male performance level. Primary performance observed on this test relates to acquired motor skill levels. It had been demonstrated that motor learning is an important variable in performing a motor task. With an increase in motor coordination, there was a significant increase in both skilled performance and strength levels. An additional variable, i.e., muscle mass, may contribute to skilled motor performance tasks when the observed activity include factors of strength and/or endurance. It is known that total muscle mass in females is less than in males, especially in the upper torso. The lowered PAE test results for female subjects indicate the need for further assessment of motor coordination functions in addition to strength and endurance observations. The purpose of this portion of the study was to assess changes in levels of motor coordination before and after the physical training program by use of electromyographic (EMG) analysis.

The relationship between PAE scores and EMG activity was observed. The study was conducted in conjunction with the procedures developed by USAMA and United States Army Research Institute of Environmental Medicine in the study entitled "Project 60."

RESULTS AND DISCUSSION OF THE RESULTS:

Surface EMG data were gathered from 30 female Project 60 participants. Measurements from biceps and triceps muscles were obtained during both maximum isometric and isokinetic strength and endurance evaluations. All data were recorded on FM magnetic tape and subjected to data reduction by a system developed for this project. The EMG data for the pre-test condition of Project 60 were digitized in root mean square (RMS) values and printed on paper tapes. It has been keypunched and is ready for statistical analysis. In order to facilitate the analog to digital conversion process, the post-test data were digitized by an in-house PDP-12 computer. These data were then transferred by way of paper tape to the Lawrence Berkeley Laboratory computer for statistical analysis. At the present time both the pre-test and post-test data are in an editing stage as a result of analog to digital conversion errors.

CONCLUSIONS: None.

RECOMMENDATIONS:

Statistical analysis should be completed for final report.

PUBLICATIONS: None.

OTHER PUBLICATIONS PREPARED BY GROUP:

1. Leibrecht, B.C., J.M. Hogan, G.A. Luz and K.I. Tobias. Donor and nondonor motivations. Transfusion 16: 182, 1976.
2. Lloyd, A.J. and J.T. Shurley. The effects of sensory/preceptual isolation on single motor unit conditioning. Psychophysiology 13: 340, 1976.
3. Weiser, P.C., D.A. Stamper and A. L. Dickenson. Psychological interactions leading to increased effort, leg fatigue, and respiratory distress during prolonged, strenuous bicycle riding. Symposium (In Press).
4. Stamper, D.A. Physiological, psychological and symptomatic factors affecting prolonged physical performance. Unpublished masters thesis, University of Colorado, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DAOE 6070	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUM'Y	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISB'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3S762772A814				001	
B. XXXXXXXXXX	62110A	3A162110A821				155	
C. XXXXXXXXXX	CARDS 114f						
11. TITLE (Precede with Security Classification Code)*							
(U) In Vitro Metabolism of RBCs Stored in CPD with 208.2mM Glucose and 2.04mM Adenine							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
002300 Biochemistry, 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 11		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				B. PRECEDING		C. FUNDS (In thousands)	
D. NUMBER* Not Applicable				76		.75	
E. TYPE:				77		.2	
F. KIND OF AWARD:				77		.75	
G. CUM. AMT.						60	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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				Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-5875			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) CPD Adenine; (U) Blood Storage; (U) ATP; (U) Glucose							
23. TECHNICAL OBJECTIVE* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Glucose levels of 138.8mM in citrate phosphate dextrose (CPD) adenine (Ad) will not maintain adenosine triphosphate (ATP) at levels greater than 2.0µM/gHb in 90% packed erythrocytes (rbc's) for 35 days of storage at 4°C. By increasing the glucose to 277.5mM in CPD Ad ATP was maintained at greater than 2.0µM/gHb for 5 weeks. It is important to determine if a smaller concentration of glucose, i.e., 208.2mM (1.5X normal) or less in CPD, supplemented with 2.04mM Ad will have sufficient glucose for maintenance of ATP at greater than 2.0µM/gHb after 35 days of storage. This work is important to the Army's continuing effort to extend the shelf-life of stored rbc's.</p> <p>24. (U) The levels of glucose, ATP, 2,3-diphosphoglycerate and pH will be determined in blood stored in the CPD.</p> <p>25. (U) 76 04 - 76 09 Maintenance of ATP at >50% of original concentration has been obtained in erythrocytes stored for 42 days when packed to 80% hematocrit in a CPD adenine (0.25mM final) and 177mM glucose (1.25X normal). This medium should provide greater than 70% viability on reinfusion of both whole blood and packed cells after 35 days of liquid storage. A military optional additive system (MOAS) has been under development to allow addition of glucose and adenine to blood up to 14 days after collection in CPD thus permitting separation of components without exposure to adenine or glucose as well as permitting 35 day storageability of erythrocytes. This MOAS should allow greater flexibility to the military in its blood banking operations by allowing production of additive-free components and yet allow up to 35 day storage of erythrocytes for reinfusion into the combat wounded soldier. Publications: Bensinger, TA. Zuck TF, Transfusion 16:353-356, 1976.</p>							

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 155 In Vitro Metabolism of RBCs Stored
in CPD with 208.2mM Glucose and 2.04mM
Adenine

The following investigations have been conducted under this Work Unit:

STUDY NO. 1 In Vitro Metabolism of Packed Erythro-
cytes Stored at Various Glucose Con-
centrations in CPD-Adenine
STUDY NO. 2 Effect of Optional Additive Systems
(OAS) on In Vitro Erythrocyte Metabolism

Study No. 1 To determine more precisely the minimum amount of adenine and glucose necessary to allow maintenance of adenosine triphosphate (ATP) at greater than 50% of original levels for 35 to 42 days of storage, studies are in progress in which the blood storage bags contain citrate-phosphate-dextrose (CPD) supplemented with 1.25x, 1.50x, or 1.75x glucose and three levels of adenine: 0.18mM, 0.25mM, or 0.37mM. Data derived from 1.25x and 1.50x glucose with all three levels of adenine revealed maintenance of ATP at greater than 50% of original for a full 35 days of liquid storage in the six CPD combinations thus far examined. At 42 days when only 1.25x glucose and 0.18mM adenine are present, ATP drops to 47% of original values.

Study No. 2 Recent emphasis on the potential complication of nephrotoxicity in soldiers massively transfused with red cells preserved with adenine has prompted investigations to develop blood storage systems with lower adenine concentrations by manipulating the timing of adenine addition. The method which allows adding adenine and extra glucose up to 14 days after original collection of CPD anticoagulated blood and blood components, has been termed the Military Optional Additive System (MOAS). Supplementation, on Day 0, 7, or 14, in 70% hematocrit packed units with as little as 0.125mM adenine (compared with 0.25mM as a minimum effective adenine concentration when added to the CPD drawing anticoagulant) and 0.25x glucose provides adequate maintenance of ATP through 42 days of storage. Therefore, either an integral MOAS system or a sterile coupling device should provide a means for supplementation of erythrocytes up to 14 days after initial collection to allow 35 to 42 day liquid storage and at the same time would permit harvesting other blood components without exposure to the additives.

BODY OF REPORT

WORK UNIT NO. 155

In Vitro Metabolism of RBCs Stored
in CPD with 208.2mM Glucose and
2.04mM Adenine

STUDY NO. 1

In Vitro Metabolism of Packed Erythro-
cytes Stored at Various Glucose Con-
centrations in CPD-Adenine

PROBLEM:

Citrate-phosphate-dextrose (CPD) has been established as a useful anticoagulant for liquid blood storage up to 21 days and, with adenine supplementation, CPD will permit storage of red blood cells for 35 to 42 days. European formulas for CPD-adenine have contained 0.50mM final concentration of adenine. Since potential renal toxicity exists for adenine, even though most clinical reports have documented its safety, it would be desirable to develop a formula for CPD which would contain the least amount of adenine that will allow storage in the liquid state for 35 to 42 days.

RESULTS AND DISCUSSION OF THE RESULTS:

McGaw Laboratories have provided blood storage bags with CPD-adenine containing 1.25x, 1.50x, and 1.75x glucose and 0.18mM, 0.25mM and 0.37mM adenine (final concentration). Thus, a total of nine different formulas were available for study. Investigations have been completed with 1.25x glucose and 0.18mM, 0.25mM, and 0.37mM in both whole blood and 80% packed cells. The 0.18mM adenine concentration provided 53% of original adenosine triphosphate (ATP) levels on Day 35 and 47% of initial ATP levels on Day 42 in whole blood. The 0.25mM and 0.37mM concentrations of adenine provided greater than 50% of initial ATP levels after 42 days of liquid storage as both whole blood and packed cells with 1.25x glucose. However, the glucose levels were depleted in some, but not all, units when packed to 80% and stored for 42 days.

CONCLUSIONS:

The amount of adenine in the primary bag containing CPD should be greater than 0.18mM when glucose is 1.25x. Only study of the other formulas will determine if levels of adenine such as 0.18mM final concentration will be effective in maintaining ATP at greater than 50% of original level if additional glucose is present.

RECOMMENDATIONS:

Studies should be complete by exploring other formulas.

PUBLICATIONS:

1. Bensinger, T. A., and T. F. Zuck: Additional studies concerning the metabolism of packed erythrocytes in CPD-adenine. Transfusion 16:353-356, 1976.

STUDY NO. 2

Effect of Optional Additive Systems
(OAS) on In Vitro Erythrocyte Metabolism

PROBLEM:

Modified preservative solutions which would safely extend useful shelf life of liquid stored red cells beyond the present 21-day limit would benefit the military significantly. However, currently, supplements are incorporated in the preservative solutions into which the whole blood is drawn. The non-red cell components, principally plasma fractions and platelets, are therefore exposed to these supplements. It is also probable that red cells transfused within the first two weeks of liquid storage may not benefit from supplements which prolong liquid storage intervals. If a technique could be developed which would allow supplements to be added at sometime after initial collection, a longer shelf life could be obtained and adverse effects of adenine minimized.

RESULTS AND DISCUSSION OF RESULTS:

Units of blood drawn into CPD were packed to 70% or 90% hematocrit within three hours of drawing and then divided into subunits which were supplemented on Days 0, 7, or 14 with adenine (0.25mM final concentration) and glucose. Many of the 70% and 90% hematocrit subunits showed a rapid increase in red cell ATP concentration by the time of supplementation. All subunits supplemented on Day 7 and 14 had equal or better ATP concentrations throughout the time in storage when they were compared with the units supplemented on Day 0. There was no significant difference between the 2,3 diphosphoglycerate, pH, and hematocrit measurements in the subunits of either group.

CONCLUSIONS:

The MOAS system can sharply reduce the potential toxicity risk of supplements, such as adenine, by: 1) decreasing the amount of adenine necessary for ATP maintenance, and 2) not exposing all components to the supplement by harvesting them soon after collection.

RECOMMENDATIONS:

Studies should be continued to determine optimal timing of addition of supplements and establish the minimal effective concentrations of each. A feasible bag configuration should be developed with interested manufacturers.

PUBLICATIONS:

1. Zuck, T. F., and T. A. Bensinger: Special report: Implications of sterile docking devices. Transfusion 15:399-401, 1975.
2. Zuck, T. F., and T. A. Bensinger: Beneficial effects on red cell ATP of delayed addition of glucose and adenine. Transfusion 15:517, 1975 (abstract).
3. Bensinger, T. A., and T. F. Zuck: Optional additive systems as an effective strategy for blood storage in military blood banking. Proceedings of the Annual Army Science Conference, West Point, New York (in press), 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9b. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3M161102BS02				075	
b. CONTRACTOR	62110A	3A162110A821				156	
c. CONTRACTOR	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Investigation of Cell-Free Resuscitating Solutions							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
008800 Life Support, 003500 Clinical Medicine, 002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 03		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER: Not Applicable				76		1.5	
c. TYPE:				FISCAL		.5	
d. KIND OF AWARD:				YEAR		25	
e. AMOUNT:				CURRENT			
f. CUM. AMT.				77		1.5	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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RESPONSIBLE INDIVIDUAL				ADDRESS: Department of Surgery			
NAME: Canham, J.E., COL, MC				Presidio of San Francisco, CA 94129			
TELEPHONE: (415) 561-3600				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
21. GENERAL USE				NAME: DeVenuto, Frank, PhD, DAC			
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				SOCIAL SECURITY ACCOUNT NUMBER:			
				ASSOCIATE INVESTIGATORS			
				NAME: Zuck, Thomas F., LTC, MC			
				NAME:		POC: DA	
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Stroma-Free Hemoglobin; (U) Blood Substitute Solutions; (U) Hemorrhagic Shock							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Stripped stroma-free hemoglobin (SFH) could provide the basis for an ideal temporary life support system for the severely wounded soldier. It is capable of <u>in vivo</u> on-loading and off-loading oxygen with sufficient efficiency to maintain normal oxygen consumption in experimental animals rendered free of circulating red cells. SFH has been found superior to electrolyte solutions in resuscitation of animals following acute hemorrhagic shock. Potentially it can be stored for extended periods without refrigeration. The object of these studies is to evaluate the practicality of SFH as a resuscitation and oxygen carrying blood substitute solution.</p> <p>24. (U) Hemoglobin prepared by crystallization as the protein base for a cell-free resuscitation solution is being evaluated in animal models for its effects on critical organ function and maintenance of morphologic integrity. Storage stability under various conditions is being studied using methemoglobin formation as an index of deterioration. Optimal concentrations of metabolites, electrolytes, and supplemental protein for improved <u>in vivo</u> function are being determined.</p> <p>25. (U) 76 04 - 76 09 Total and partial exchange transfusions of hemoglobin solutions in rodents have shown superior maintenance of vital signs, improved survival, and superior preservation of liver, kidney, and brain morphologic integrity compared with control rodents exchanged with albumin solutions. The elimination pattern of ⁵¹Cr-labeled hemoglobin has been studied in rats, and the one-half disappearance time determined. Using glycerol as a stabilizer, crystalline hemoglobin has been lyophilized and reconstituted with only minimal increased methemoglobin formation.</p>							

DD FORM 1 MAR 68 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery

WORK UNIT NO. 156 Investigation of Cell-Free Resuscitating Solutions

Hemoglobin, prepared by crystallization, has been used as a blood substitute in total (91-93%) and partial (70-76%) blood replacement studies. Exchange transfusions have been carried out in laboratory animals (rats) to a total blood replacement of 91-93% with hemoglobin or with albumin solutions. When albumin was used, all animals died at approximately ten minutes after transfusion was completed, whereas all animals transfused with hemoglobin survived for five hours and displayed normal activity during this time. In these studies the plasma half-disappearance time of hemoglobin was 3.5 hours and body distribution of ^{51}Cr -labeled hemoglobin, as a percent of initial levels, has shown 6% in the kidney, 6% in the liver, 10.5% in the marrow, and 13-14% in the urine at 3 hours after transfusion.

Survival was obtained with all animals transfused with hemoglobin or albumin solutions to a partial blood replacement of 70-76%. However, the oxygen capacity of the circulating fluid in the hemoglobin transfused animals was about three times greater than that found in the corresponding albumin-transfused control. Values of hemoglobin, hematocrit, platelets, and P_{50} returned to normal pretransfusion levels within five to seven days.

Light microscopy morphological studies have shown that transfusion with hemoglobin solution does not cause detectable adverse effects on kidney, brain, or liver cells.

Hemoglobin solutions have been lyophilized in the presence of glycerol with little increase in methemoglobin formation following reconstitution.

BODY OF REPORT

WORK UNIT NO. 156

Investigation of Cell-Free Resuscitating Solutions

PROBLEM:

Having a solution readily available which is capable of transporting oxygen and maintaining oncotic pressure has multiple advantages, particularly when massive transfusions are required. Plasma, dextran, albumin, and other preparations have been used. Although they appear to be effective as plasma expanders, they have limited use as blood substitutes since they do not carry oxygen. As an oxygen carrying resuscitating fluid, blood has a limited storage life and requires typing and cross-matching.

In most civilian clinical settings in this country, the transfusion requirements associated with trauma can be met with conventionally stored blood and its components. However, military needs frequently demand massive fluid support in areas remote from supply sources. These situations present uniquely difficult storage and transportation problems. Predicting the time and place when field transfusion requirements may suddenly increase further complicates fluid therapy logistics. If a stable solution, capable of carrying and exchanging oxygen, could be stockpiled, many of these difficulties could be avoided.

Hemoglobin is a protein which can transport and exchange oxygen, has oncotic activity, can be prepared from outdated blood, does not require typing and cross-matching, can be stored for long periods of time under sterile conditions, and has low viscosity. However, if hemoglobin is used as a blood substitute, it is imperative that it be free of any stromal particles, stromal lipid, or other soluble and insoluble cell components (which have been implicated in adverse effects on kidney function and on coagulation). Hemoglobin has the potential to provide the basis for an ideal life-support system for the severely wounded soldier. Developing an effective blood substitute is pertinent not only to military combat casualties, but also to the civilian population which would incur many casualties in a mass disaster.

RESULTS AND DISCUSSION OF THE RESULTS:

A rapid, simple and reproducible method has been developed in our laboratory for the preparation of hemoglobin solution. The method,

based on a crystallization procedure, yields hemoglobin free of soluble and insoluble red blood cell components. The hemoglobin thus prepared is also free of group A and B activity, does not affect conventional coagulation tests, and has been used for infusion to replace blood in laboratory animals.

Exchange transfusions have been carried out in rats to a total blood exchange of 91-93% and to a partial blood exchange of 70-76% with hemoglobin or albumin (control) solutions serving as blood substitutes.

Total Blood Exchange. The results of total blood exchange using hemoglobin or albumin solutions in two groups of four rats each show that at the end of blood exchange the hematocrit decreases to between 3 and 4% from a pre-exchange level of 42-43% (i.e., a 91-93% blood replacement). The hemoglobin concentration in the hemoglobin transfused animals decreases to 46% of the initial value, whereas the hemoglobin concentration for the albumin-transfused rats decreases to 9%. Values of pH are not affected by infusion of hemoglobin or albumin solution, whereas platelet counts drop sharply both in the experimental and control groups. In the albumin transfused rats the P_{50} values show a slight decrease and no changes are observed in the n-values; in the hemoglobin transfused animals a considerable decrease in the P_{50} and slightly lower n-values are obtained due to dilution of residual red cells with the hemoglobin solution. However, at the end of transfusion, the oxygen content of the circulating fluid in the experimental animals is five times greater than the oxygen content observed in the control group; the range of volume of oxygen was 9.5 to 10.4 cc per dl for hemoglobin-transfused animals and 1.4 to 1.8 cc per dl for albumin-transfused animals. All control rats, transfused with albumin, died approximately 10 minutes after transfusion; the rats transfused with hemoglobin engaged in (apparently) normal activity during a five-hour survival period.

Plasma Disappearance of Hemoglobin. In an effort to learn the reason why the hemoglobin-transfused animals survived only five hours, the plasma disappearance of hemoglobin was studied. The results demonstrate that plasma disappearance of ^{51}Cr -radiolabeled hemoglobin is linear with time with a rate calculated to be $15.2 \pm 1.1 \mu\text{g}$ per 100 ml per minute. Half-disappearance time, for the net doses administered in these studies, was 3.5 hours. These rates were observed in three rats by determination of plasma hemoglobin concentration and by calculation of the disappearance in percent of initial concentration. By extrapolating the line at five hours, only about 30% of the initial hemoglobin concentration remained in the plasma.

Partial Blood Exchange. In other experiments, two groups of four rats have been transfused with hemoglobin or albumin solution to a partial blood replacement of 70-76%. Survival of all animals was obtained. Hematocrit, total hemoglobin, pH, platelet count, P_{50} , n-values, and O_2 content were monitored during the post-transfusion period up to two weeks. Although significant differences are present between the hemoglobin-transfused and the control groups, all parameters return to pretransfusion levels within five to eight days. However, during the immediate post-transfusion period and also in the following days, the oxygen content of the circulating fluid in the hemoglobin transfused animals was greater than that found in the corresponding albumin infused controls.

Tissue Distribution of ^{51}Cr -radiolabeled hemoglobin. In the total transfusion studies, urinary excretion of hemoglobin was monitored. At three hours after transfusion, 13-14% of the hemoglobin present in the rat at the end of transfusion was excreted in the urine. Since the hemoglobin content of the urine (13-14%) and plasma (53-59%) at three hours after transfusion did not account for all the hemoglobin infused in the rats, the tissue distribution was studied using hemoglobin labeled with ^{51}Cr . The results of the distribution of radiolabeled material in a group of three rats transfused to 91% blood replacement and sacrificed at three hours after transfusion showed that the radioactive material, as a percent of initial levels, was present in significant amounts in kidneys (6%), liver (6%), marrow (10.5%), urine (13-14%), and in the intravascular fluid (56%). In another experiment in which two rats were transfused to a 66% blood replacement with ^{51}Cr hemoglobin and sacrificed at 24 hours after transfusion, radioactive material was still present in some of these organs. As percent of initial values, kidneys contained 2.9%, liver 3.6%, and marrow 8.1%, urine 30%; no radioactivity could be determined in plasma or red blood cells.

Preparation of Hemoglobin from Sources Other than Human Blood. In the preparation of hemoglobin, soluble and insoluble red blood cell components are removed including phosphate compounds, such as 2,3-diphosphoglycerate (2,3-DPG), which influences the oxygen binding affinity. Human blood with intact fresh red cells has a P_{50} of about 27 mm Hg, whereas hemoglobin, as prepared by the crystallization procedure, demonstrates a P_{50} of 15-18 mm Hg.

Crystallized bovine hemoglobin has been investigated. Such hemoglobin has been prepared by the same procedure described for human blood; the solutions have been analyzed. The absorbance spectra (between 350 and 650 nm) of human and bovine hemoglobin are identical and

represent oxyhemoglobin, with absorption maxima at 414, 540, and 576 nm and absorption minima at 510 and 560 nm. In the two hemoglobin preparations, essentially the same values were obtained for methemoglobin content, pH, and volume percent of oxygen. However, the P_{50} of crystallized bovine hemoglobin was greater (28.5 mm Hg) than that of crystallized human hemoglobin (16.3 mm Hg).

Possible Effect of Transfusion of Hemoglobin on the Morphology and Function of Several Organs. Several studies have been initiated in cooperation with MAJ H. Friedman, Department of Surgery, and CPT P. O'Mara, Department of Biomedical Stress, to ascertain whether or not transfusions with hemoglobin solutions cause detrimental morphologic or psychologic changes or adverse effects. In one phase of these studies, laboratory animals were transfused with hemoglobin or albumin solutions to different blood replacement levels. Groups of animals were sacrificed at several post-transfusion periods and the various organs fixed by perfusion. Slices observed by light microscopy for possible morphological changes indicate that transfusion with hemoglobin solution does not cause detectable adverse effects on kidney, brain, or liver cells. In another phase of these studies, groups of rats have been trained to perform specific tasks. These animals have been transfused with hemoglobin or albumin solution to 70-75% blood replacement and the retention of training levels has been monitored for several days after transfusion. The results obtained thus far are inconclusive, but cerebral impairment has been suggested.

Lyophilization of Hemoglobin Solution. It would be desirable for logistics and storage purposes to reduce the hemoglobin solution to a powdered form; sterile water would be added to obtain a solution at the time needed for transfusion. The addition of glycerol prevents the transformation of hemoglobin to methemoglobin during the process of lyophilization. The powder thus obtained is easily reconstituted to hemoglobin solution by adding water equivalent to that lost during the freeze-drying process.

CONCLUSIONS:

Hemoglobin, prepared by crystallization, has been used for total and partial blood exchange in the rat. As a blood substitute, hemoglobin appears to be beneficial in restoring and maintaining vital signs and does not cause detectable adverse effects in the light microscopy morphology of liver, kidney, and brain cells. Complete survival and return to normalcy is observed with blood replacement of 70-76%. Plasma disappearance of infused hemoglobin is linear with time and significant amounts of hemoglobin are present in kidney, liver, and

marrow. Hemoglobin has been prepared by crystallization from sources other than human blood. Hemoglobin solutions have been lyophilized in the presence of glycerol without significant increase in met-hemoglobin formation.

RECOMMENDATIONS:

Preparation of hemoglobin by the method of crystallization, as developed in our laboratory, should be contracted with interested biological manufacturers in order to obtain large quantities of hemoglobin solution necessary for clinical studies in higher animals. These studies will permit monitoring of important physiological, biochemical, and hematological parameters. Investigation of possible effects of hemoglobin solution on kidney, liver, lung, and brain cells should be continued, in order to exclude any potential toxicity which might occur during and after transfusion.

The development of hemoglobin from animal sources should be explored for their potential as laboratory solutions. Efforts to prepare hemoglobin in a powder form need to be intensified. If this were accomplished problems of supply, storage, and transport would be reduced substantially.

PUBLICATIONS:

1. DeVenuto, F., W. Y. Moores, A. I. Zegna, and T. F. Zuck. Characteristics of stroma-free hemoglobin (SFH) prepared by crystallization. Transfusion 15: 525, 1975. Presented at the annual meeting of the American Association of Blood Banks, November 1975.
2. DeVenuto, F., S. M. Wilson, T. A. Billings, and C. E. Shields. In vivo distribution of injected ^{14}C -dioxymethylene in tissues and organs of normal rats. Transfusion 16: 24, 1976.
3. DeVenuto F., and S. M. Wilson. Distribution of progesterone and its effect on human blood during storage. Transfusion 16: 107, 1976.
4. DeVenuto, F., T. F. Zuck, A. I. Zegna, and W. Y. Moores. Characteristics of stroma-free hemoglobin prepared by crystallization. J. Lab. Clin. Med. (in press), 1976.
5. DeVenuto F., A. I. Zegna, W. Y. Moores, and T. F. Zuck. Transfusions with hemoglobin prepared by crystallization. Proc. Army Science Conf. (in press), 1976. Presented at the Army Science Conference, West Point, N. Y., June 1976.

6. DeVenuto, F., T. F. Zuck, W. Y. Moores, and A. I. Zegna. Blood exchange in the rat with hemoglobin prepared by crystallization. Transfusion (in press), 1976. To be presented at the 1976 annual meeting of the American Association of Blood Banks, San Francisco, November 1976.

7. DeVenuto, F., W. Y. Moores, A. I. Zegna, and T. F. Zuck. Total and partial exchange in the rat with hemoglobin prepared by crystallization. Transfusion (in press), 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL		
10. NO. CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62772A		3S762772A814		002	
b. COMMON		62110A		3A162110A821		157	
c. COMMON		CARDS 114f					
11. TITLE (Precede with Security Classification Code) ^a							
(U) In Vitro Metabolism of G6PD Deficient Erythrocytes Stored in CPD Adenine Anticoagulant							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 03		78 03		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER ^a Not Applicable				76		.3	
c. TYPE:				FISCAL YEAR		7T	
e. KIND OF AWARD:				CURRENT		.1	
f. CUM. AMT.				77		.3	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				ADDRESS ^a Blood Research Division			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME ^a Bensinger, Thomas A., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-5875			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Blood Storage; (U) Adenine; (U) CPD; (U) G6PD							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) Ten percent of the American Negro male population is heterozygous for Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, and this population makes a significant contribution to the blood donation program of the U.S. Army. Hence, military blood banks may contain a significant amount of G6PD deficient blood. This study will determine the ability of G6PD deficient erythrocytes to maintain metabolic integrity during 35 days of storage in CPD-II (CPD adenine with 1.25X glucose) as measured by levels of ATP, GSH, as well as Heinz body formation.							
24. (U) Blood will be drawn from identified G6PD deficient donors and stored in the CPD-II anticoagulant media at 4C. At appropriate time intervals, samples will be removed aseptically from these bags, and tests for ATP, GSH and Heinz body formation will be undertaken. Additionally, the ability of glucose isotopically labeled in the C-1 position to be converted to CO ₂ and ribose through the hexose monophosphate shunt will be determined.							
25. (U) 76 04 - 76 09 A total of seven units of G6PD deficient blood have been stored in a variety of anticoagulant media including CPD, CPD adenine, CPD ascorbate, and CPD adenine ascorbate. G6PD deficient cells do not respond as well to adenine in maintaining ATP as do nondeficient erythrocytes; however, they do possess higher ATP levels in comparison to G6PD deficient cells not stored in adenine media. Ascorbate does appear to promote 2,3-DPG maintenance in G6PD deficient cells as it does in nondeficient cells. An additional finding is an excess accumulation of pyruvate in G6PD deficient blood when stored in all four anticoagulants when compared to normal controls in the same storage media. This increased pyruvate may result from decreased NADPH levels in G6PD deficient cells. NADPH is a necessary cofactor for LDH when the pH decreases during storage.							

^aAvailable to contractors upon originator's approval.

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 157 In Vitro Metabolism of G6PD Deficient
Erythrocytes Stored in CPD Adenine
Anticoagulant

The following investigations have been conducted under this Work Unit:

- STUDY NO. 1 Measurement of ATP, 2,3-DPG, pH, GSH
and Glucose Levels of G6PD Deficient
Blood Stored in CPD and CPD Adenine
- STUDY NO. 2 Measurement of Some Intermediate Meta-
bolites and Enzyme Activity of G6PD
Deficient Erythrocytes Stored in CPD,
CPD Adenine Ascorbate Anticoagulant
Media when Compared to Normal Non-
deficient Erythrocytes Stored in the
Same Media

Study No. 1. See Annual Progress Report for FY 1975.

Study No. 2. Five units of blood from glucose-6-phosphate dehydro-
genase (G6PD) deficient individuals have been stored in four different
anticoagulant media and compared to five units drawn from normal Black
controls and stored in the same manner. Assays of various metabolic
intermediates and enzyme activities including glucose-6-phosphate,
lactate, pyruvate, catalase, reduced glutathione (GSH), and methemo-
globin have revealed a significant difference only in levels of pyru-
vate in the G6PD deficient cells in comparison to the normal controls.
This increase in the pyruvate levels may reflect decreased levels of
NADPH available in G6PD deficient cells. Because NADPH is a necessary
cofactor for lactic acid dehydrogenase (LDH) as pH decreases, the
pyruvate may accumulate more readily in the G6PD deficient cells.
Additional studies are underway to attempt to elucidate this finding.

BODY OF REPORT

WORK UNIT NO. 157 In Vitro Metabolism of G6PD Deficient Erythrocytes Stored in CPD Adenine Anticoagulant

STUDY NO. 1 Measurement of ATP, 2,3-DPG, pH, GSH, and Glucose Levels of G6PD Deficient Blood Stored in CPD and CPD Adenine.

This study was summarized in the Annual Progress Report for FY 75, pages 159-160.

STUDY NO. 2 Measurement of some Intermediate Metabolites and Enzyme Activity of G6PD Deficient Erythrocytes Stored in CPD, CPD Adenine, CPD Ascorbate and CPD Adenine Ascorbate Anticoagulant Media When Compared to Normal Nondeficient Erythrocytes Stored in the Same Media

PROBLEM:

Since 10% of the American Negro male population is deficient for glucose-6-phosphate dehydrogenase (G6PD), it is important to determine if erythrocytes from these donors behave in the same manner metabolically as erythrocytes from normal controls when stored under identical conditions. Since approximately 25-30% of all military personnel are Black, the possible adverse effects of G6PD deficient blood donors could be especially severe in the U.S. Army. The newer additives, such as adenine or ascorbic acid, to prolong liquid storage to 35-42 days could cause additional stress of G6PD deficient blood. Therefore, this study was undertaken to evaluate what, if any, changes do occur during six weeks of storage.

RESULTS AND DISCUSSION OF THE RESULTS:

Blood from five G6PD deficient individuals was drawn into divided subunits of 125 ml of whole blood. Each subunit contained either citrate-phosphate-dextrose (CPD), CPD-adenine, CPD-ascorbate, or CPD-adenine-ascorbate. Samples of blood drawn from Black donors not G6PD deficient were used as controls. Subunits were combined with the same anticoagulants and were stored and manipulated identically. All subunits were stored at 4°C and analyzed weekly for pH, adenosine triphosphate (ATP), 2,3-DPG, glucose-6-phosphate, reduced glutathione (GSH), lactate, pyruvate, catalase and methemoglobin. Pyruvate levels

were increased threefold by 42 days of storage in the G6PD deficient blood compared to the control blood. There were no differences in the lactate levels in the two groups. This change in lactate/pyruvate ratios may reflect a lack of NADPH available as a cofactor for lactic acid dehydrogenase (LDH) to convert pyruvate to lactate. This becomes pronounced in the G6PD deficient cells only when pH drops and the K_m of LDH for NADPH decreases.

CONCLUSIONS:

Further studies should be undertaken to elucidate this pyruvate accumulation and to see if increased pyruvate is detrimental to G6PD stored erythrocytes.

RECOMMENDATIONS:

None

PUBLICATIONS:

1. Bensinger, T. A.: Prolonged maintenance of 2,3 diphosphoglycerate (2,3-DPG) in glucose-6-phosphate dehydrogenase (G6PD) deficient stored blood. Clinical Research 24:107A, 1976 (abstract).
2. Bensinger, T. A. and F. Medina: Excessive pyruvate accumulation in G6PD deficient (G6PD-Def) erythrocytes during liquid storage. Accepted for presentation at the 19th Annual Meeting of the American Society of Hematology, Boston, Mass., December 7, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISB'D INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3S762772A814				003	
B. CONTRACTOR	62110A	3A162110A821				158	
C. CONTRACTOR	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Preservation of 2,3-DPG in Red Cells Subjected to Extended Liquid Storage							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
008800 Life Support, 003500 Clinical Medicine, 002300 Biochemistry, 002600 Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 03		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
D. NUMBER ^a Not Applicable				76		1.5	
C. TYPE:				77		.4	
E. KIND OF AWARD:				77		2.0	
F. CUM. AMT.						36	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				ADDRESS ^a Blood Research Division			
				Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME ^a Zuck, Thomas F., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-4611			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Bensinger, Thomas A., LTC, MC			
				NAME: Moore, Gerald L., PhD, DAC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Blood Storage; (U) 2,3-DPG; (U) Hemoglobin Function							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) Although the significance at the tissue level of a left-shifted oxygen dissociation curve exhibited by 2,3 diphosphoglycerate (DPG) deficient red cells is not completely clear, it is highly probable that superior red cell function would improve organ function in the massively transfused, acutely wounded soldier. Currently used red cell storage anticoagulant-preservative solutions (APS) do not permit the red cell to maintain DPG concentrations. Normal oxygen off-loading characteristics of hemoglobin are dependent, at least in part, on the allosteric regulation of DPG within the red cell. These studies will develop and evaluate both additive and storage systems which will enable red cells to better maintain DPG during liquid storage, without impairing other glycolytic intermediates such as adenosine triphosphate (ATP).							
24. (U) Most APS additives which currently appear to hold promise for improving red cell DPG during storage are unstable in the anticoagulant mix. Among these are dihydroxyacetone (DHA), ascorbic acid (AA), and bicarbonate (BC). Stable forms or systems employing these substances will be developed and tested. The stable forms must be inert to the plastic blood container, freely convertible to the active form upon contact with whole blood, and nontoxic both to the blood in storage and the recipient on infusion.							
25. (U) 75 05 - 76 09 A stable form of ascorbate, ascorbate 2-phosphate, has been found to tolerate autoclaving and retain activity following prolonged storage in aqueous media. In combination with DHA, it improves red cell DPG concentration during storage. Two commonly used DPG assays have been compared to determine precision and accuracy. Stability studies and assay techniques for DHA are in progress.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 158 Preservation of 2,3-DPG in Red Cells
Subjected to Extended Liquid Storage

The following investigations have been conducted under this Work Unit:

STUDY NO. 2 Stabilization of Ascorbic Acid as a Red Cell Preservative
STUDY NO. 4 The Evaluation of Analytical Methods for 2,3-DPG in Stored Blood
STUDY NO. 5 The Stabilization of Dihydroxyacetone in CPD Solution for Use in Maintaining Red Cell 2,3-DPG During Liquid Storage

All currently available techniques for the liquid preservation of red cells do not maintain the red cell 2,3 diphosphoglycerate (2,3-DPG) concentration sufficiently to allow hemoglobin to release oxygen optimally. This defect of stored red cells is of great importance in hemorrhagic shock, and, thus, in resuscitation of combat wounded.

Study No. 2 It has been found that ascorbate 2-phosphate will maintain stored erythrocyte 2,3-DPG as well as the unstable sodium ascorbate, and that it works synergistically with dihydroxyacetone to improve red cell 2,3-DPG maintenance. By using an assay developed in this laboratory, ascorbate 2-phosphate has been found fairly stable under conditions required for blood bank containers: it has maintained 80% of activity after autoclaving and appears to be stable for 3 to 4 months when stored at room temperature.

Study No. 4 Because published reports of 2,3-DPG concentration of stored red cells were at variance, comparative studies of the fluorometric assay and the spectrophotometric assay were undertaken. The precision, accuracy, time and cost factors of the two procedures were compared. The fluorometric assay exhibits higher accuracy and precision while being faster and less costly.

Study No. 5 Although dihydroxyacetone (DHA) is known to promote 2,3-DPG maintenance in red cells during storage, DHA is unstable in anticoagulant solutions. Because of its red cell preservation value, its properties were studied. Two colorimetric assays for methylglyoxal (MGA), the primary breakdown product of aqueous DHA, were examined and thin layer chromatography separation of MGA and DHA was developed. Long-term storage of DHA has been started in various solutions and anticoagulants.

BODY OF REPORT

WORK UNIT NO.	158	Preservation of 2,3-DPG in Red Cells Subjected to Extended Liquid Storage
STUDY NO.	2	Stabilization of Ascorbic Acid as a Red Cell Preservative

PROBLEM:

After 14 days of conventional liquid storage as whole blood or packed cells, erythrocyte 2,3-diphosphoglycerate (2,3-DPG) is not maintained at levels permitting normal oxygen transfer to tissues. Data are emerging which suggest that decreased oxygen transfer may be particularly deleterious to soldiers requiring massive transfusions to correct hemorrhagic shock. It has recently been found that ascorbic acid, especially in combination with dihydroxyacetone, will maintain 2,3-DPG levels greater than 50% of original concentrations for 28 to 35 days of liquid storage. However, ascorbate, either as the acid or as the sodium salt, is not stable in solution and decomposes rapidly when exposed to the autoclaving necessary to sterilize blood bags. It is important, therefore, to discover ways of either stabilizing ascorbic acid itself, or to develop new compounds which have the same potential effect as ascorbic acid in preserving red cell 2,3-DPG. These compounds must be stable enough to withstand through rigors of autoclaving and withstand storage prior to utilization at phlebotomy. Thus, investigations are needed to stabilize ascorbic acid or find stable substitutes for ascorbic acid.

RESULTS AND DISCUSSION OF THE RESULTS:

Since our previous work showed that it was impractical to maintain solutions of ascorbic acid levels for longer than 3 to 4 weeks in the plastic bags, work has been done to identify a stable form. Such a substitute has been found in ascorbate 2-phosphate, a phosphorylated form of ascorbic acid. An assay has been developed for this compound by using alkaline phosphatase to cleave the stabilizing phosphate radical. Ascorbate 2-phosphate tolerates autoclaving, i.e., 80% of its activity is maintained after autoclaving at 121°C for 20 minutes in solution. Ascorbate 2-phosphate also has been found to be stable when stored for 4 to 6 months at room temperature in either plastic or glass bottles with no attempt to exclude oxygen. It appears to be stable in a pH range of 5 to 8. Ascorbate 2-phosphate has been found to be as active as ascorbic acid or sodium ascorbate in maintaining red cell 2,3-DPG concentrations, especially when utilized in association with dihydroxyacetone.

CONCLUSIONS:

Ascorbate 2-phosphate appears to be a practical additive to maintain red cell 2,3-DPG during liquid blood storage.

RECOMMENDATIONS:

Stability studies should be completed, and the effects of ascorbate 2-phosphate on red cell 2,3-DPG should be analyzed.

PUBLICATIONS:

None

STUDY NO.	4	The Evaluation of Analytical Methods for 2,3-DPG in Stored Blood
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PROBLEM:

The analysis of red blood cell 2,3 diphosphoglycerate (2,3-DPG) levels is an important parameter in blood storage studies designed to improve the functional quality of banked blood. This is of particular military importance in studies of stored blood since it is widely held that soldiers in hemorrhagic shock should be transfused with red cells of optimal oxygen carrying capacity. Several assays for 2,3-DPG are available, but these yield somewhat varying results from the several laboratories investigating the functional properties of stored blood. Two assays used in this laboratory were compared as to their analytical quality and time-cost limitations.

RESULTS AND DISCUSSION OF THE RESULTS:

A comparison study has been done between the fluorometric end point assay of Keitt (as modified in this laboratory) and the spectrophotometric rate assay of Krinsky (as modified by Beutler). A double blind experiment was done in which various amounts of standardized 2,3-DPG solutions were added to blood aliquots followed by multiple analysis with each assay procedure. The mean values on a given sample were compared against the standard added value and shown to be statistically different. The average absolute error was 2.0% for the fluorometric assay and 12.6% for the spectrophotometric assay. Accuracy and precision were shown to be higher for the fluorometric assay, while time per sample and reagent costs were shown to be lower than the spectrophotometric procedure.

CONCLUSIONS:

The spectrophotometric assay yields results that are adequate when used as a screening procedure of functional properties of stored blood. When experiments are done requiring the highest accuracy and precision of 2,3-DPG measurement, the fluorometric assay is to be preferred.

RECOMMENDATIONS:

None

PUBLICATIONS:

1. Ledford, M. E., and G. L. Moore: A comparison of two 2,3-diphosphoglycerate assays. Manuscript in review.

STUDY NO. 5

The Stabilization of Dihydroxyacetone
in CPD Solution for Use in Maintaining
Red Cell 2,3-DPG During Liquid Storage

PROBLEM:

Dihydroxyacetone (DHA) is effective in improving 2,3-diphosphoglycerate (2,3-DPG) maintenance in stored red cells. In the acute resuscitation from hemorrhagic shock due to combat injuries, red cells of optimal oxygen transport characteristics should be transfused. However, DHA cannot be introduced into the blood bag with aqueous anticoagulants since it decomposes during bag storage and when autoclaved in these solutions. It is necessary to study the rate and mechanism of DHA decomposition in various aqueous solutions and find a method of blocking this decomposition (to methylglyoxal). Stabilization of DHA may be achieved by development of a complex or derivative, termed DHA-X. The stabilized DHA-X should be autoclavable and storable in aqueous solution for three years.

RESULTS AND DISCUSSION OF THE RESULTS:

The breakdown of DHA in aqueous solutions for form methylglyoxal (MGA) is slow in pure water, but accelerated by certain ions such as phosphate, borate, or organophosphates. Two colorometric assays have been set up to detect MGA generation in DHA solutions. Both involve formation of a 2,4-dinitrophenol hydrazine derivative, and both have the shortcoming of giving a background with DHA. A thin layer chromatography system has been developed to measure and separate DHA and MGA, but it requires larger than usual amounts

of sample due to the low detectability of these compounds. A peroxide oxidation assay has been developed to quantitate standard solutions of MGA. Initial studies have been done to confirm the observation that the DHA breakdown is accelerated in the presence of high concentrations of certain ions such as phosphate. Long term storage studies have been started on DHA dissolved in water, saline, citrate-phosphate-dextrose (CPD), adenine glucose solution, and sodium phosphate. These are being monitored by pH, UV scanning spectrophotometry, thin layer chromatography, and the MGA assays. No change has been seen after 1 month at 25°C.

CONCLUSIONS:

None

RECOMMENDATIONS:

These studies should be continued to develop a stable form of DHA.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 75 07 01	4. KIND OF SUMMARY H. Termination	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8A. DISSEM INSTR* NL	8B. SPECIFIC DATA CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62110A	3A162110A821		00		159	
B. CONTRIBUTING							
C. XXXXXX	CARDS 114 f						
11. TITLE (Precede with Security Classification Code)* (U) Optimal Storage Conditions for Liquid Platelet Concentrates							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine, 012900 Physiology, 008800 Life Support							
13. START DATE 75 03		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				B. PRECEDING 76 7T		C. FUNDS (in thousands) 5 30	
B. NUMBER: Not Applicable				FISCAL YEAR CURRENT		0 2	
C. TYPE:				77		0	
D. KIND OF AWARD:				0		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				Blood Research Division			
				Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Zuck, Thomas F., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-4611			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Bensinger, Thomas A., LTC, MC			
				NAME: Bucci, Thomas, LTC, VC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Platelet Storage (U) Thrombocytopenic (U) Platelet Metabolism (U) Platelet Function							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) Not infrequently platelet transfusion support is adjunctive to massive red cell infusions in response to trauma. Field availability of viable platelets has been severely hampered by their limited tolerance to storage and transportation. Optimal liquid storage systems have not been established. The objective of these studies is to develop storage techniques which would make platelets more readily available in the field. 24. (U) Platelet concentrates from human and subhuman primates will be stored under various conditions. Platelet morphology, metabolism, function, and bacteriocidal properties will be assessed. <u>In vivo</u> survival and function will be evaluated in subhuman primates rendered experimentally thrombocytopenic. 25. (U) Because of the highly variable metabolic behavior in this laboratory of individual concentrates subjected to liquid storage, and increasingly pessimistic literature reports of extending the storage interval for liquid platelet concentrates with existing equipment, this work unit has been terminated and efforts directed to developing methods of cryopreservation of platelets for field use (see Work Unit 042). Further studies on the effects of increased glucose in anticoagulant solutions, if required, will be performed in conjunction with red cell metabolism studies (Work Unit 155).							

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 159 Optimal Storage Conditions for
Liquid Platelet Concentrates

The following investigations have been conducted under this Work Unit:

It became clear early in the fiscal year, from both published reports of other workers and studies under this work unit, that current acceptable liquid storage duration for platelets could probably be only extended minimally. Even a doubling of the current 96 hours maximum effective period would be inadequate for platelet support of soldiers injured OCONUS if supply was required from within CONUS. With these realizations in mind, the decision was made to develop strategies for frozen storage of platelets. Further, an animal model for studying platelet function following transfusion has been published by other workers. The employment of antibodies to induce thrombocytopenia is more promising than the filtration methods attempted under this work unit. Therefore, this work unit is terminated and efforts redirected to study cryopreservation in an animal model (rabbit) employing antibody to induce thrombocytopenia (see Work Unit 042).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL		
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3S762772A814			004		
b. SECONDARY	62110A	3A162110A821			160		
c. THIRDARY	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^a							
(U) CPD-Adenine Clinical Trials							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine, 008800 Life Support							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 01		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER: Not Applicable				76		2.0	
c. TYPE:				FISCAL		.5	
d. KIND OF AWARD:				77		107	
e. CUM. AMT.				CURRENT			
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Blood Research Division			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Zuck, Thomas F., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-4611			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Bensinger, Thomas A., LTC, MC			
				NAME: Peck, Carl C., LTC, MC POC: DA			
23. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Blood Transfusion (U) Adenine (U) CPD (U) Blood Storage (U) Military Blood Banking							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) The objective of clinical trials of an improved anticoagulant is Food and Drug Administration licensure, which would permit clinical use of red cells after prolonged liquid storage.							
24. (U) Current red cell liquid storage is limited to 21 days. Data derived through use of an anticoagulant-preservative solution containing CPD, adenine, and additional glucose, suggest the storage period can be extended to 35 days, and postinfusion red cell survival would be superior at any given day of storage. Since shipment of blood into combat areas necessitates delays between drawing and infusion, the impact of these delays on the quality of red cells infused will be minimized through use of such a solution. A cooperative effort of military and civilian investigators will perform clinical trials under an application for investigational new drug (I.N.D.) held by the container-solution manufacturer. The Blood Research Division, LAIR, is the cooperative effort team leader. Data from each study will be collated and analyzed by this division in conjunction with the Department of Information Sciences.							
25. (U) 75 07 - 76 09 Using blood containers from two manufacturers with active I.N.D.s, autologous stored red cell viability has been studied by four investigators. Twenty-four hour postinfusion 51-Cr red cell survivability of red cells stored for 35 days as whole blood or packed cells in CPD-adenine was determined. The results were nearly identical to data published for cells stored 21 days in CPD or ACD. Recently, these data have been presented to the Bureau of Biologics efficacy panel, which unanimously recommended that applications for licensure for CPD-adenine as an anticoagulant for red cells be favorably considered.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery

WORK UNIT NO. 160 CPD-Adenine Clinical Trials

This work unit was initiated pursuant to a request from the United States Army Medical Research and Development Command that a prolonged liquid anticoagulant be made available for field use. Because of the extensive interfacing of military supply lines with civilian blood sources, it was decided by this unit that a licensure effort would be instituted. A multilaboratory task force was organized by the Blood Research Division, LAIR, to perform the clinical studies necessary for licensure. Two manufacturers, using protocols, report forms, and safety summaries developed under this work unit, filed investigational new drug applications (IND). Approval was gained by reviewing authorities in the Federal Drug Administration, Bureau of Biologics, to proceed with Phase IA (red cell survivabilities in normal donors) of the IND. Phase IA is essentially completed, with four laboratories throughout the nation participating, and LAIR acting as lead laboratory. The red cell survivability data for both whole blood and packed red cells stored for 35 days in CPD-adenine containing bags from two manufacturers are essentially identical to reported survivabilities for these components stored in CPD and ACD for 21 days. These data were presented to the efficacy panel of the Bureau of Biologics which unanimously recommended that CPD-Adenine with supplemental glucose be approved as an anticoagulant for all blood bank products with the exception of platelet preparations.

BODY OF REPORT

WORK UNIT NO. 160

CPD-Adenine Clinical Trials

PROBLEM:

Over the past fifteen years, efforts have been directed at prolonging shelf-life of red cells for transfusion by fortifying acid-citrate-dextrose (ACD) anticoagulant solution with purine bases (adenine, inosine, guanine). It has become apparent from many studies that adenine is effective and accompanied by rather low risks of toxicity. Much of this work was performed by the Blood Research Division at Fort Knox, Kentucky, and by Army contractors. Military logistics require extended storage of red cells, particularly to support transfusion requirements in remote areas during hostile activities. However, despite millions of units of blood containing adenine which have been transfused in Europe, extended liquid storage is not available for military use. Clinical trials were needed. Previous civilian efforts to institute clinical trials were not successful for reasons unrelated to the scientific merits of the anticoagulant. With the advent of a national blood policy, it became clear that interface of civilian and military donor resources would be essential to meet sudden large requirements. Thus, general licensing of an adenine-fortified anticoagulant would be necessary if the military were to use it most effectively. Efforts were initiated under this work unit to obtain general Food and Drug Administration (FDA) licensure, with the realization that even should this not be successful, data would be available to permit use in those unique military settings in which extended liquid blood storage might be essential. If the data suggested lack of safety and efficacy, then further Army funding of adenine blood research would require careful scrutiny.

RESULTS AND DISCUSSION OF THE RESULTS:

During the period of this report, progress has been made:

1. Two container anticoagulant manufacturers (Fenwal Laboratories and McGaw Laboratories) have filed investigational new drug applications (IND) which were approved by nonobjection of the Bureau of Biologics (BoB). The protocol was also approved by the R&D Command in Washington, including human use aspects of the licensing efforts. In conversations held with Dr. Lewellys Barker of the BoB, it was made clear that, until Phase IA (red cell survivability in normal donors) of the IND protocol was completed, and the survivability proven satisfactory, the balance of the clinical studies could not be commenced.

2. During the fourth quarter, FY 1976, four laboratories (The American Red Cross, Dr. Paul McCurdy; Harvard University, Dr. Lawrence Button; City of Hope, Dr. Ernest Beutler; Blood Research Division, LAIR, LTC Thomas Bensinger) instituted the Phase IA survivability studies as provided in the protocol. The initial survivabilities of packed red cells performed in the laboratory of Dr. Beutler were disappointing; however, subsequent studies done by other laboratories suggested that the results were essentially identical following 35 days of packed cell storage, as those for packed red cells stored for 21 days in CPD or ACD.

In Phase IA studies of whole blood stored in CPD-adenine with supplemental glucose for a period of 35 days, the mean autologous survivability of red cells 24 hours postinfusion was 80.69 ± 6.57 (SD) in 32 normal donors, both male and female. Four of these units had been subjected to the trauma of transportation by shipping by air from one coast to the other and then returned to the originating laboratory for survivability studies. This mean survival and standard deviation compare favorably with comparable studies reported for red cells stored in CPD for 21 days (mean survivability $74.9\% \pm 6.43\%$ (SD) in 38 male and female normal recipients). Similarly, the data compare favorably with the mean survivability in 41 normal male and female recipients of ACD whole blood stored for 21 days (77.8% mean survivability $\pm 6.24\%$ standard deviation). These studies demonstrate that the survivability of CPD-adenine red cells stored as whole blood for 35 days are at least as good as, and perhaps slightly superior to, currently licensed CPD and ACD cells stored as whole blood for 21 days.

The Phase IA studies of packed red cell survivabilities showed a mean survivability 24 hours after infusion of $71.02 \pm 10.0\%$ (SD) in 11 normal male and female donors. This compares favorably with the single large series of 21-day stored packed cells, in which the survivabilities were $70.1\% \pm 11.5\%$ (SD) in 70 normal recipients (ACD), and $72.6\% \pm 11.8\%$ (SD) in 21 normal donors (CPD). Thus, although the packed red cell survivabilities are not quite as high as predicted from in vitro red cell adenosine triphosphate measurements, and the standard deviation suggests that numerous units will survive less than 70%, the CPD-adenine anticoagulated packed red cells stored for 35 days are at least as good as packed cells stored in currently licensed ACD or CPD for 21 days.

In all of the above studies, there were no apparent differences among the various laboratories, with the exception of a slightly lower mean packed cell survivability in Dr. Beutler's laboratory.

Similarly, the units subjected to transportation showed no adverse effect on their survivability, and there was no difference apparent among the different bag manufacturers.

3. During a recent meeting held by the Bureau of Biologics at the National Institutes of Health in Washington, DC, the efficacy panel for blood and blood products heard summaries of available data concerning adenine supplementation from a wide variety of presenters. This laboratory presented data on the toxicity of adenine, the disposition of diethylhexyl phthalate as it is leached into blood products, and the results of the Phase IA task force discussed in paragraph 2 above. It was the unanimous recommendation of this panel that a new drug application by the blood container manufacturers for adenine and supplemental glucose fortified CPD should be favorably considered by the Bureau of Biologics and the Federal Drug Administration. It was recommended that it should be approved for all products, with the exception of platelets, since little platelet data are available concerning the efficacy when prepared from blood in adenine supplemented anticoagulant.

CONCLUSIONS:

CPD-adenine with 0.25mM adenine and additional glucose supplementation is effective in preserving red cells as whole blood for 35 days, and probably effective for packed red cells for a similar length of time.

RECOMMENDATIONS:

1. Additional studies should be performed to complete Phase IA as drafted to study tightly packed red cells to ensure that the 11 units completed are indeed representative.
2. Participate in those platelet studies required to document their efficacy and safety if prepared from CPD-adenine supplemented with additional glucose to permit licensing for all liquid stored blood products currently licensed for CPD and ACD.
3. Participate with the manufacturers in applying for a new drug application, and apply through the Surgeon General's office for the required amendment to the institutional blood bank license held by the Surgeon General to permit the Army as dictated by military requirements to use CPD-adenine as formulated in this study.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3S762772A814				011	
B. CONTRACTOR	62110A	3A162110A821				161	
C. CONTRACTOR	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Problems in Abdominal Trauma							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine, 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 01		78 06		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				B. PRECEDING		b. FUNDS (In Thousands)	
B. NUMBER ^a Not Applicable				76		.75	
C. TYPE:				FISCAL YEAR		7T	
D. AMOUNT:				CURRENT		.2	
E. KIND OF AWARD:				77		.75	
F. CUM. AMT.						38	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Div of Combat and Experimental Surg Department of Surgery Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Friedman, Harold I., MAJ, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3115			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS Geasling, T.W., CPT, VC			
				NAME: Moores, William Y., LTC, MC			
				NAME: Gardiner, Barry N., LTC, MC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Abdominal Trauma; (U) Small Bowel Physiology; (U) Liver Morphology							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Data from the Vietnam Conflict indicate that the abdomen is the third most frequently wounded anatomical region. The high fatality rate (47.1%) of soldiers undergoing side-to-side small bowel anastomosis during WW II has been attributed to the massive amount of intestine resected in these cases. The long term squellae associated with massive resection have made surgeons cautious at the initial repair. The objective of these studies is to develop small bowel neomucosa on transposed stripped colonic wall as an autologous graft to cure the short bowel syndrome associated with massive small bowel resection following abdominal trauma.</p> <p>24. (U) Initial morphologic studies will define the hepatic and small bowel adaptive changes following massive resection using tissue from patients undergoing intestinal bypass procedures as experimental models. These changes will be correlated with those seen in similarly treated animals, which will then be subjected to replacement of the resected small bowel with stripped colonic wall to study the absorption capacity of the colonic neomucosa.</p> <p>25. (U) 76 04 - 76 09 In preliminary studies, segments of colon were stripped of all inner layers to the outer circular muscle and were placed in continuity in the jejunum. Considerable fibrosis occurred and limited growth of neomucosa. Subsequently, similar segments with removal of only epithelium and lamina propria were used. This treatment is expected to decrease fibrosis and improve reepithelization. Patients undergoing the bypass procedure displayed marked hepatic fat infiltration. The percentage of lipid in hepatic cytoplasm, its effect on cellular organelles, and the reversibility of steatosis were documented. The pathologic significance of intramitochondrial filaments in association with hepatic fat infiltration was also documented.</p>							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery

WORK UNIT NO. 161 Problems in Abdominal Trauma

The following investigations have been conducted under this Work Unit:

STUDY NO. 1 The Short Bowel Syndrome

Large portions of small intestine may require resection following extensive gunshot and missile wounds. Since an insufficient functional absorptive area to transport nutrient material may remain, a considerable threat to life may result. Increasing the absorptive surface by converting a transposed segment of large intestine into small intestine offers a potential solution. Under this work unit, patients undergoing intestinal bypass procedures were used as a model to study small bowel and liver adaptation following massive loss of absorptive surface. Several new findings by light and electron microscopy suggest that intramitochondrial filament changes in liver cells represent an adaptation to increased stress, but are reversible.

The response of transposed colonic segments in mongrel dogs has also been studied. The normal colonic mucosa of the transposed segment must be denuded in order to permit absorptive epithelium to regenerate, and this has proven technically difficult. It was determined that removing structures deeper than the epithelium and lamina propria results in fibrosis and shortening of the transposed segment. Although restricting the mucosal stripping to superficial layers is technically more tedious, it has resulted in improved viability of transposed segments.

BODY OF REPORT

Work Unit No. 161 Problems in Abdominal Trauma

Study No. 1 The Short Bowel Syndrome

Ex-1 Analysis of functional activity of neo-small-bowel
mucosa in dogs

PROBLEM:

The complications of removing large portions of small intestine secondary to trauma, such as gunshot and missile wounds in the abdomen, pose considerable threat to patient life. The primary defect is an insufficient functional absorptive area to transport nutrient material. A potential solution involves increasing the absorptive surface area by converting a transposed segment of large intestine into small intestine.

RESULTS AND DISCUSSION OF THE RESULTS:

Seven mongrel dogs underwent mobilization of a 10 cm segment of proximal colon with its mesenteric vascular supply intact. The epithelium, lamina propria, muscularis mucosa, and submucosa were removed. Three animals did not survive. At autopsy, infarction of the segment and massive peritonitis were evident. Surviving animals underwent reexploration at one to three month intervals following surgery. All four animals displayed contraction of the segment to approximately 1 cm with massive inflammatory reaction and scar formation. In one small area, however, neomucosa forming apparent glands or crypts had appeared to bridge the distance between adjoining jejunum. It was proposed that if fewer layers of colonic wall were removed, the inflammatory reaction would be reduced, and re-epithelization of the denuded colon would be more satisfactory. Therefore, in three of the four surviving animals, a new 10 cm colonic segment was mobilized, and only the epithelium and lamina propria were removed. These three animals have survived this procedure to date and will be re-explored in approximately three months. In addition, several chemical agents are currently being evaluated for their ability to remove only the epithelial cells, and thereby leave the lamina propria intact.

CONCLUSIONS:

Removal of epithelium, lamina propria, muscularis mucosa, and submucosa from the colonic wall results in an inflammatory reaction which inhibits growth of jejunal neomucosa onto the denuded colonic

surface. Removal of fewer layers (i.e., only epithelium and lamina propria) from the colonic wall may provide less inflammatory reaction, and a better surface for reepithelization.

RECOMMENDATIONS:

After jejunal neomucosa formation is confirmed the segment will be tested for its ability to transport nutrient material. Finally, animals which have undergone massive small bowel resections will receive a denuded colonic segment, and its ability to improve the functioning absorptive surface will be analyzed to determine whether this approach will lessen morbidity following massively resected small bowel necessitated by combat trauma.

Ex-2 The intestinal bypass procedure for morbid obesity as an experimental model of acute intra-abdominal trauma requiring extensive small intestine resection

PROBLEM:

To provide effective management for soldiers who have undergone massive small bowel resections following combat trauma requires a thorough understanding of the adaptive changes which occur in the remaining intestine and liver after the traumatized bowel is resected. Patients with intestinal bypasses to control obesity were selected as the model. Liver and intestinal alterations following the procedure were investigated. Attention was focused on several alterations which have been observed in experimental animals following massive intestinal resections. In addition, attempts were made to clarify the ultrastructural mechanism of hepatic fatty infiltration. Intramitochondrial filaments have been described in association with a variety of disease states of the liver, including fatty metamorphosis. They have also been observed in "normal" livers. Determining the origin of the filaments, their significance, and prevalence in disease states versus normal livers was one aspect of the study.

RESULTS AND DISCUSSION OF THE RESULTS:

Fourteen obese patients underwent an intestinal bypass procedure in which 12 inches of jejunum was anastomosed to 6 inches of ileum. Preoperatively, intestinal biopsies were obtained in both the fasted and fat-fed states from each patient. An open liver biopsy was obtained at the time of surgery. Postoperatively, capsule intestinal and percutaneous liver biopsies were harvested at 6-month intervals. Three control patients underwent open-liver biopsies at the time of exploratory laparotomy for disease primarily unrelated to the liver.

All patients in the study, including one control patient, displayed varying degrees of hepatic fatty infiltration at surgery. Lipid was predominantly localized in pericentral cells, and the intracellular lipid content diminished in cells closer to the portal triad. Pericentral cells also displayed an increase in total cross-sectional surface area of three to five times that of hepatocytes near the bile duct. Preliminary studies also indicated that accumulation of large quantities of lipid within the liver cells displaced the cytoplasm peripherally and crowds the organelles. Organelle concentration within the cytoplasm is increased, but the lipid did not appear to damage the organelles morphologically.

Following the bypass procedure, liver steatosis increased during the first 6 months. This increase was reflected in a high percentage of cells near the bile duct accumulating large lipid droplets. Subsequently, there was a gradual clearing of lipid from the hepatocytes.

At the ultrastructural level, hepatocytes of 13 of 14 patient displayed intramitochondrial filaments. These inclusions primarily were concentrated in periportal cells. Control patients, lacking steatosis, exhibited few or no filaments. A number of observations suggest that the filaments arise from mitochondrial cristae. Following the bypass procedure and improvement in the degree of steatosis, the filaments either decreased in incidence or were no longer observed. These data suggest that the filaments are related to the altered hepatic metabolic state which induces fatty metamorphosis. The biochemical nature of the filaments has not yet been determined.

A study on the morphology of intestinal fat absorption using morphometric techniques was completed. This investigation demonstrated changes in cellular organelles and Golgi complex during fat absorption in the rat and will be used as a basis for analyzing the ultrastructural alterations in absorptive cells of humans following the intestinal bypass procedure.

CONCLUSIONS:

All patients undergoing the intestinal bypass procedure for obesity displayed fatty infiltration of the liver predominantly oriented around the central vein. Following bypass surgery, the degree of steatosis worsens and then gradually improves. Cellular lipid accumulation does not morphologically damage the intracytoplasmic organelles but, rather, concentrates them in the peripheral cytoplasm surrounding the lipid. Intramitochondrial filaments in the hepatocytes occur in association with fatty infiltration and are derived from mitochondrial cristae. The incidence of filaments in

hepatocytes of control subjects is insignificant. The filaments most likely represent a response to an altered liver metabolic state.

RECOMMENDATIONS:

Evaluation of the cellular response of the small intestine and liver should be continued and correlated with the findings in dogs in Study 1.

PUBLICATIONS:

1. Friedman, H. I. and R. R. Cardell: Alterations in the morphology of the endoplasmic reticulum and Golgi complex in rat intestinal epithelial cells during fat absorption and after termination of this process. Anat Rec 184(3), p 406, 1976 (abstract).
2. Friedman, H. I. and R. R. Cardell: Alterations in the endoplasmic reticulum and Golgi complex of intestinal epithelial cells during fat absorption and after termination of this process: A morphological and morphometric study. Anat Rec (accepted for publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^c	6. WORK SECURITY ^d	DAOE 6094	76 10 01		
76 04 02	D. Change	U	U	NA	NL	7b. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO. / CODES ^e	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3S762772A814				005	
b. SECONDARY	62110A	3A162110A821				162	
c. THIRDARY	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^f							
(U) Evaluation of DEHP (di-2-ethylhexylphthalate) Disposition in Primates (Including Man)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^g							
016800 Toxicology, 012600 Pharmacology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07		77 09		DA		C. In-House	
17. CONTRACT / GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES / EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER ^h Not Applicable				76		2.0	
c. TYPE:				FISCAL YEAR		0.2	
e. KIND OF AWARD:				CURRENT		15	
				77		64	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Blood Research Division			
				Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Peck, Carl C., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-5875			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Odom, Daniel G., CPT, MS			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Di-2-ethylhexylphthalate (DEHP); (U) Blood Storage Containers; (U) Plasticizer							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To determine the distribution, biotransformation and elimination characteristics of DEHP in primates (including man) as a step towards assessment of the potential for human toxicity of this contaminant of blood storage in polyvinylchloride (PVC) bags. (PVC bags are currently the method of choice, in the military, for transport and storage of blood.)</p> <p>24. (U) Following development of a sensitive and specific assay for DEHP and its metabolites, the dependence of DEHP leaching on hematocrit and plastic surface area exposed, time, and temperature will be determined. The pharmacokinetics and metabolism of DEHP will be determined in the monkey and man.</p> <p>25. (U) 76 04 - 76 09 A sensitive, specific, and accurate gas chromatographic assay for DEHP has been developed. Factors affecting DEHP leaching into plasma during blood storage are being incorporated into a kinetic model. Pharmacokinetics and metabolism of ¹⁴C-DEHP infused into African Green Monkeys have been established and will serve as a basis for further studies of DEHP disposition in Man.</p>							

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 162 Evaluation of DEHP (di-2-ethylhexyl phthalate) Disposition in Primates (Including Man)

The following investigations have been conducted under this Work Unit:

STUDY NO. 1 DEHP Assay Development
STUDY NO. 2 Factors Affecting DEHP Leaching
STUDY NO. 5 Pharmacokinetic Evaluation of DEHP

Increasing the storage duration for whole blood and packed red cells will improve the logistics of field transfusion therapy. The plasticizer diethylhexyl phthalate (DEHP) leaches from polyvinyl chloride (PVC) plastic containers into the plasma during storage, and concern has been expressed concerning its potential toxicity in transfusion recipients. Since DEHP leaching from PVC increases linearly with time, prolonging storage increases potential toxicity. To properly assess the increased risk, detailed studies of leaching characteristics, the factors affecting leaching rates, and pharmacokinetic evaluation of DEHP are essential.

A simplified and less costly chromatographic assay for DEHP in biological materials has been developed and standardized. This procedure compares favorably in terms of recovery, precision, and accuracy to previously used methods. Studies on the effects of time, temperature, hematocrit, and plastic surface area exposed to blood on DEHP leaching are in various stages of completion. A kinetic model of these effects are under development.

A pharmacokinetic evaluation of DEHP disposition and metabolism in the African Green Monkey has been completed. Radiolabeled DEHP, leached into autologous plasma from plastic strips and then reinjected, disappears rapidly from the plasma. The clearance is predominantly renal. Preliminary evaluation of human DEHP pharmacokinetics has been evaluated in studies of patients receiving large amounts of DEHP in platelet concentrates.

BODY OF REPORT

WORK UNIT NO. 162

Evaluation of DEHP (di-2-ethylhexyl phthalate) Disposition in Primates (Including Man)

STUDY NO. 1

DEHP Assay Development

PROBLEM:

Prolonging the liquid storage duration of whole blood and packed red cells is one of the primary missions of this department. Red cell outdating poses a major problem in combat medical supply logistics, and this problem could be solved, in part, by the availability of extended storage. During liquid storage of red cells for transfusion, the plasticizer, diethylhexyl phthalate (DEHP) leaches from the polyvinyl chloride (PVC) plastic storage container into the plasma. The rate of DEHP accumulation in stored plasma is linear with time, and thus, higher concentrations are observed during extended storage intervals. Concern has been expressed concerning the potential toxicity of DEHP in transfusion recipients, although current data of toxic effects are not satisfactory. If it is to be recommended that the shelf-life of whole blood and packed red cells be extended during combat operations, the potential risks of increasing the dose of DEHP must be evaluated accurately. Under this work unit assay techniques, leaching characteristics, and pharmacokinetics of DEHP are being evaluated.

Initially, an assay system had to be established. Previously used procedures employed a thin layer chromatography (TLC) step to separate DEHP from plasma. Although this step was known to be extremely costly, it was employed to prevent fouling of the gas chromatography (GC) columns with plasma constituents. The TLC step itself was a source of error due to the possibility of contamination of the test system with DEHP, a compound almost ubiquitous in the environment. To reduce both reagent and time costs, an assay was sought which did not require a TLC step.

RESULTS AND DISCUSSION OF THE RESULTS:

The modified GC DEHP assay omitting the TLC step has proven satisfactory. The recovery from plasma samples was $81 \pm 5\%$ with a precision of 5%. The lower limit of quantitative sensitivity was 100 ng per ml plasma and the lower limit of qualitative detectability of approximately 50 ng per ml of plasma. The assay has been successfully used for all of the studies under this work unit. Although

precise cost accounting is difficult, the assay is less than one-half as expensive as those employing TLC. All risks of DEHP contamination from the TLC plates have been eliminated. In practice, the GC column has required replacement due to fouling only every several months (at a cost of less than \$50.00).

CONCLUSIONS:

The direct GC assay of DEHP in plasma without a TLC step is accurate, precise, sensitive, and less costly than previous techniques which included a TLC step. The assay is satisfactory for leaching studies.

RECOMMENDATIONS:

The assay should be routinely applied in leaching studies, and modified further as required for metabolic and pharmacokinetic studies in man and lower primates.

PUBLICATIONS:

None

STUDY NO. 2

Factors Affecting DEHP Leaching

PROBLEM:

Developing ways to limit human exposure to DEHP (in plasma) requires identification of factors affecting DEHP leaching into plasma from polyvinylchloride (PVC) blood bags. This will permit selection of storage conditions which minimize leaching.

RESULTS AND DISCUSSION OF THE RESULTS:

DEHP accumulation is apparently linear during the first 42 days of storage at 4°C. There is no significant difference in DEHP leaching rates between Travenol PL-146 and McGaw BB-69 blood bags. A small amount of plasma mono-ethylhexyl phthalate accumulates (greater in BB-69 than in PL-146 blood bags) during storage. Increasing hematocrit results in somewhat higher plasma DEHP concentrations but lower total DEHP leached per blood unit. Long-term storage studies suggest a curvilinear accumulation profile, the kinetic parameters of which bear an unexpected temperature dependence.

CONCLUSIONS:

The effects of time, temperature, and plastic area exposed on rates of DEHP leaching have been determined, and the effects of red cell concentration on total DEHP leached have been quantitated.

RECOMMENDATIONS:

Studies of the factors affecting DEHP leaching suggest that a comprehensive kinetic model should be developed which incorporates the essential factors: time, temperature, hematocrit, and exposed plastic surface area.

PUBLICATIONS:

1. Odom, D., J. Brady, D. Jess, C. Peck, and P. Albro: Plasticizer leaching. (manuscript in preparation)
2. Peck, C., J. Bailey, B. Barrett, and D. Odom: A kinetic model of DEHP migration into plasma from polyvinylchloride bags. (manuscript in preparation)

STUDY NO. 5

Pharmacokinetic Evaluation of DEHP

PROBLEM:

The pharmacokinetics and metabolic fate of DEHP following intravenous infusion of plasma containing leached DEHP is unknown in nonhuman primates and man. In order to assess risks of toxicity in massive transfusions, these parameters must be known.

RESULTS AND DISCUSSION OF THE RESULTS:

^{14}C -DEHP leached into autologous plasma from polyvinylchloride (PVC) strips was intravenously infused into three African Green Monkeys. Serial plasma, urine, and stool samples were analyzed for ^{14}C and ^{14}C metabolites of DEHP (GC-mass spectroscopy). Plasma ^{14}C concentrations were fit to a triexponential equation for elucidation of disappearance half-lives and clearance. Metabolic studies revealed eleven DEHP metabolites appearing in urine. Preliminary studies of urine from humans receiving large doses of intravenous DEHP via platelet transfusions are underway.

CONCLUSIONS:

Distribution and elimination of ^{14}C from ^{14}C -DEHP is rapid and metabolic conversion to conjugated species which appear in urine is extensive.

RECOMMENDATIONS:

These data yield a basis for planning studies which should be undertaken to establish DEHP pharmacokinetics in man.

PUBLICATIONS:

1. Peck, C. C., F. J. Bailey, D. G. Odom, H. E. Blatt, and B. B. Barrett: Plasticizer disposition in a conscious primate. *Pharmacologist* 18:195, 1976. Presented at Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, New Orleans, LA, 17 August 1976.
2. Peck, C. C., F. J. Bailey, D. G. Odom, H. E. Blatt, and B. B. Barrett: Plasticizer kinetics in a subhuman primate species. *Transfusion*, in press. Accepted for presentation at the annual meeting, American Association of Blood Banks, San Francisco, November 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DAOE 6077	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING	8. DISSEM INSTRN	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
						WORK UNIT NUMBER	
a. PRIMARY		62772A		3S762772A814		012	
b. CONTRACTING		62110A		3A162110A821		163	
c. CONTRACTING		CARDS 114f					
11. TITLE (Precede with Security Classification Code)*							
(U) Studies in Pulsatile Extracorporeal Circulation							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
008800 Life Support, 016200 Stress Physiology, 009800 Medical and Hospital Equipment							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 11		77 06		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		2.0	
b. NUMBER* Not Applicable				FISCAL YEAR		25	
c. TYPE				CURRENT		87	
d. KIND OF AWARD:				77		2.0	
e. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Div of Combat and Experimental Surg Department of Surgery Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME* Moores, William Y., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3385			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hannon, J.P., PhD, DAC			
				NAME: Rodkey, W., CPT, VC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U)Pulse Pressure (U)Combat Surgery (U)Trauma (U)Wet Lung Syndrome (U)Pulsatile Perfusion (U)Left Ventricular Function (U)Oxyhemoglobin Diss							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) The objective is to develop a subprimate model appropriate for total extracorporeal circulation and permit precise measurements of ventricular hemodynamic and metabolic function. This model will be used to conduct extracorporeal perfusion with various modalities of pulsatile flow, and modified animal model for subsequent use with transfusion products of differing oxyhemoglobin dissociation characteristics.							
24. (U) An extracorporeal circulation system capable of supplying whole body perfusions with pulsatile flow at various pulse pressures has been developed. Left ventricular function studies will be done to determine the effects of this perfusion on myocardial function. Radioactive labeled microspheres will be used for coronary flow distribution measurements.							
25. (U) 75 07 - 76 09 A cardiovascular investigational laboratory has been established for accurate measurements of pressure, flow, cardiac output, and ejection fraction. An initial canine study has failed to show any beneficial effect of pulsatile perfusion on preservation of myocardial function. Metabolic studies with coronary flow distribution studies in swine have also failed to show a beneficial effect with pulsatile perfusion for the myocardium during beating, fibrillating, working, and nonworking situations. Initial investigations into the role of the oxyhemoglobin dissociation curve variation and myocardial performance have begun with in vitro manipulation of P ₅₀ in swine blood, and the technical performance of rapid exchange transfusions using the manipulated blood in the extracorporeal perfusion circuit. Publications: Chatterjee K, Tyberg J, Stowe D, Ratshine R, Moores W, et al. Circ. 51, 52 (suppl II):168, 1975.							

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 163 Studies in Pulsatile Extracorporeal Circulation

The following investigation is being conducted under this Work Unit:

STUDY NO. 1 The Effect of Variation in Pulse Pressure on Left Ventricular Function in the Dog and Swine

Study No. 1 The purpose of this study is to test the hypothesis that pulsatile perfusion in an extracorporeal life support system is superior to continuous perfusion in preserving myocardial function. The pulsatile mode permits direct control of heart rate, stroke volume, and afterload for controlled measurements of left ventricular function. Myocardial metabolism and coronary flow distribution can be measured with arterial-coronary sinus blood sampling and injection of radioactive labeled microspheres. The initial canine study with 12 satisfactory perfusions showed small decreases in myocardial function in all animals but these decreases were not related to perfusion modality. Coronary flow distribution and oxygen consumption measurements also failed to demonstrate any beneficial effect of pulsatile perfusion. Coronary flow measurements indicated that pulsatile coronary flow dynamics were present in the coronary artery of the beating heart even with continuous aortic root perfusion.

The relationship between preservation of myocardial performance and P_{50} values of the priming solutions is being investigated in the swine isolated heart preparations. These studies are designed to determine whether the P_{50} of resuscitation fluids, including whole blood, is a significant determinant of recovery from hemorrhagic shock secondary to massive combat wounds.

BODY OF REPORT

WORK UNIT NO. 163

Studies in Pulsatile Extracorporeal Circulation

STUDY NO. 1

The Effect of Variation in Pulse Pressure on Left Ventricular Function in the Dog and Swine

PROBLEM:

In designing an extracorporeal circulation system for prolonged life support in the injured combat soldier, there is controversy about the benefit or necessity of pulsatile flow. Evidence for the direct effect of pulse pressure on left ventricular function in such a system is not available. Because of the technical complexity of pulsatile perfusion, continuous perfusion would be preferred in field settings. We are investigating the effects of pulsatile perfusion on the preservation of left ventricular function, and are attempting to discern its importance in an acute life support system. Previous work in this study indicated a possible effect of an abnormally shifted hemoglobin dissociation curve on preservation of myocardial function. An additional aim of this study is to determine the effect of P_{50} variation of solutions used for acute resuscitation from combat injuries.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary experiments established the animal model preparation, tested the pulsatile perfusion system, and confirmed the validity of left ventricular function measurements. The initial study involving 12 dogs has been completed. This study compared pulsatile and non-pulsatile support in preserving myocardial integrity. Animals in both the pulsatile and continuous flow groups showed mild depression in values for stroke work and stroke volume, with no decreases in dp/dt or coronary flow; however, different perfusion modalities (pulsatile or continuous) did not show different effects on these values. Myocardial lactate extraction was higher in the pulsatile group with a slightly decreased oxygen consumption; possibly this indicates a minor metabolic advantage for pulsatile perfusion.

The dogs in the study had wide variation in their P_{50} values (oxygen dissociation), and although these variations seemed to correspond with levels of myocardial performance, the correlation was not consistent, and the exact effect of P_{50} could not be derived.

Since the results from the canine study showed a wide variation among animals, it was proposed that the study could be improved with a

different model, one which had fewer variances between individuals of the same breed. Swine were chosen for this reason. Also, in swine, oxyhemoglobin dissociation is dependent on red cell 2,3 diphosphoglycerate (2,3-DPG) concentration (it is not dependent in dogs). This characteristic of swine, coupled with the known similarity to human cardiovascular physiology, should make the use of scarce nonhuman primates unnecessary.

Two series of experimental runs with swine have been completed. These series have concentrated on coronary flow dynamics with pulsatile and continuous flow in conjunction with variation in myocardial rhythm (beating versus fibrillation) and working conditions (loaded versus vented ventricle). Myocardial flow distribution has been determined with radioactive labeled microspheres. Coronary flow to the epicardium and to the more crucial endocardium were measured during the various conditions. Although rhythm changes corresponded with changes in epi-endocardial flow ratio, the perfusion modality (pulsatile or continuous) had little effect. Myocardial oxygen consumption, total coronary flow, and lactate extraction were not effected by perfusion modality.

This study has concentrated on the effects of perfusion modality on myocardial performance and flow and has failed to show any advantage with pulsatile perfusion. Effects from pulsatile perfusion in the total body (decreased peripheral resistance with increased oxygen consumption), which have been noted by other investigators, were not noted in the canine study.

Initial experiments with swine have established its appropriateness as a model for left ventricular function measurements and for exchange transfusion with solutions and blood of differing P_{50} values. This portion of the study has required the in vitro preparation of swine blood with a sufficiently wide range of P_{50} that its effects on myocardial contractility can be tested. We are presently able to prepare swine blood with P_{50} values ranging from 20 mm Hg to 55 mm Hg. This blood has been successfully transfused into experimental animals with resultant in vivo P_{50} values that approach the in vitro range.

CONCLUSIONS:

This study demonstrates in two different experimental animals that, compared with continuous perfusion, pulsatile extracorporeal perfusion has little to offer in terms of preservation of myocardial performance and coronary flow.

The effect of variations in P_{50} on myocardial performance and flow has not yet been determined. However, the animal model developed to test the effect of perfusion modality on myocardial performance is appropriate for discerning the significance of the P_{50} of solutions used to resuscitate wounded soldiers in the field.

RECOMMENDATIONS:

The importance of pulsatile perfusion in a total life support system for the combat injured soldier should be examined with studies that differentiate between myocardial and total body effects of pulsatile perfusion.

The effect of changes in P_{50} on myocardial performance initially examined in the present study should be extended. The significance of the P_{50} of resuscitation solutions suitable for field use should be determined for various aspects of critical organ function.

PUBLICATIONS:

1. Chatterjee, K., J. Tyberg, D. Stowe, R. Ratshin, W. Moores, J. Ostlund, and W. Parmley: The use of ventricular endocardial QRS amplitude as a new technique for monitoring changes in left ventricular volumes. *Circulation* 51-52 (supplement II):168, 1975 (abstract).
2. Moores, W. Y., J. P. Hannon, J. Tyberg, J. D. Crum, W. G. Rodkey, D. C. Willford, and W. W. Parmley: Effects of pulsatile and continuous extracorporeal perfusion on the left ventricular function of the dog. Presented at the Fifth Meeting, Association of Army Cardiology, 13-15 May 1976.
3. Antopol, M. R., W. Y. Moores, and A. C. Gomez: The surgical treatment of an iatrogenic dissecting aneurysm of the right coronary artery. Presented at the Fifth Meeting, Association of Army Cardiology, 13-15 May 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DAOE 6098	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUMM'Y	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9b. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3S762772A814				006	
B. XXXXXXXX	62110A	3A162110A821				165	
C. XXXXXXXX	CARDS 114f						
11. TITLE (Precede with Security Classification Code) ^a							
(U) The Toxicity of Adenine When Used in Blood Anticoagulant Solution							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
016800 Toxicology, 012600 Pharmacology, 008800 Life Support							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07		77 06		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
B. NUMBER: Not Applicable				76		1.5	
C. TYPE:				FISCAL YEAR		79	
D. AMOUNT:				CURRENT		22	
E. KIND OF AWARD:				77		1.5	
F. CUM. AMT.						93	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
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				Department of Surgery			
				Presidio of San Francisco, CA 94129			
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TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-5875			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Peck, Carl C., LTC, MC			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Adenine; (U) Blood Preservation; (U) 2,3 Deoxyadenine; (U) Nephrotoxicity							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To gather further data relevant to the infusion of adenine containing blood to the massively transfused soldier. To that end, data are to be generated on the metabolism and toxicity of adenine when used as part of an anticoagulant solution for the storage of human blood. Investigations will be conducted to determine if 2,8 dioxadenine, a catabolic metabolite of adenine, could accumulate in sufficient quantities in the massively transfused individual to cause toxicity problems.</p> <p>24. (U) The rate of diffusion of adenine (when added at day 0, 7 or 14) into and out of red cells during cold storage and the rate of conversion of this adenine to nucleotides is being documented. The kinetics of adenine catabolic metabolism by xanthine oxidase will be evaluated. The rate of conversion of adenine to nucleotides and 2,3-DOA in a monkey model at various doses and chronic infusion rates is being tested.</p> <p>25. (U) 76 04 - 76 09 The pH dependent crystallization properties of 2,8-DOA in water and urine have been evaluated and indicated a solubility in water of about 2 mg/l and in urine of 4 mg/l in the physiological pH range. Adenine added to freshly drawn blood was shown to reach a plasma-red cell equilibrium in several minutes and decrease slowly thereafter, with 10% remaining after 42 days. Unmetabolized adenine is capable of egress from the red cell in response to changes in concentration gradients. A high pressure liquid chromatography system has been developed for evaluation of purine and nucleotide levels in blood. Publications: Moore, G.L., Ledford, M.E.: A modified fluorometric assay for adenine in plasma and urine. Biochem. Med. 14:147, 1975.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 165 The Toxicity of Adenine When Used
in a Blood Anticoagulant Solution

The following investigations have been conducted under this Work Unit:

STUDY NO. 1 Solubility and Crystallization Properties of Adenine and its Metabolites
STUDY NO. 2 Diffusion of Adenine Into and Out of Red Blood Cells During Cold Storage
STUDY NO. 3 Adenine Metabolism and Kinetics in CPD-Adenine Stored Blood

Study No. 1 The in vitro solubility of 2,8 dihydroxyadenine (DOA) in human urine has been determined, and apparent supersaturability demonstrated. In vivo urinary concentrations of DOA and 8-hydroxyadenine (8-OA) were measured in urine from humans receiving oral adenine. In one case, DOA concentrations far exceeded that predicted from in vitro DOA solubility studies. Study of the kinetics of DOA "apparent supersaturability" in human urine has commenced.

Study No. 2 When units of CPD blood are drawn containing 17.5 mg of adenine, about half the adenine immediately diffuses into the red cells. During 4°C storage, the plasma adenine levels slowly fall as adenine is metabolized e.g., at 21 days of storage, 3.5 mg of adenine remain in the plasma and at 42 days of storage, about 1 mg of adenine remains in the plasma.

Study No. 3 The effect of anticoagulant phosphate concentration on the rate of adenine uptake is being evaluated. In order to analyze data on adenine metabolites, a high pressure liquid chromatographic system was developed for rapid micro-quantitation of blood purines and nucleotides.

BODY OF REPORT

WORK UNIT NO. 165

The Toxicity of Adenine When Used
in a Blood Anticoagulant Solution

STUDY NO. 1

Solubility and Crystallization Prop-
erties of Adenine and Its Metabolites

PROBLEM:

The primary adverse effect of intravenously administered adenine is nephrotoxicity secondary to 2,8 dihydroxyadenine (DOA) and 8-hydroxyadenine (8-OA) crystal formation in renal tissues. Wounded soldiers in hemorrhagic shock who require massive transfusions of blood preserved by adenine supplementation may be particularly susceptible to this nephrotoxicity because of poor renal perfusion accompanying shock. In order to assess the role of adenine induced nephrolithiasis, an understanding of the in vivo urinary solubility and crystallization properties of the oxymetabolites of adenine is necessary.

RESULTS AND DISCUSSION OF RESULTS:

A UV spectrophotometric assay for DOA in buffer solutions was developed which depends on bringing the pH of the final solution to a pH region (<0) in which the absorbance is independent of the pH of the original sample.

A uniform and rigid criterion for solubility was developed which established that ultracentrifugation at 100,000 g for 10 minutes is equivalent to filtration through a .22 μ Milipore filter.

DOA solubility in various buffers (pH .25-11.4) at 37°C was established. These data were fitted to a general equation relating solubility to pH by nonlinear regression. By this technique, estimated parameters established the basal solubility ($\approx 9 \times 10^{-6} M$) and three dissociation constants. Buffer supersaturation was achieved in classic boiling experiments as well as via introduction of DOA solubilized in weak base. Maximum supersaturation exceeded basal solubility approximately 18 times, but was unstable.

Assay for DOA in urine was developed utilizing a combination of column separation and UV spectrophotometry. Basal solubility in urine was found to be slightly less than twice that of buffer. The pH fluctuations in the physiologic range for urine (pH 5-8) result in only modest changes in DOA solubility.

In vitro urine DOA solubility studies via introduction of DOA solubilized in weak base revealed enhanced ability to achieve and maintain DOA in a supersaturated state. DOA concentrations roughly ten times the basal urine solubility were easily maintained at 37°C agitated gently for up to 16 hours.

The applicability of the in vitro urine supersaturation studies to the in vivo situation was tested in a baboon administered 5 mg/Kg of adenine intravenously. DOA in solution in the three hour post-infusion urine sample approximated quite closely with the maximal in vitro supersaturated DOA concentration.

The in vitro solubility of 8-OA in human urine was studied also by means of a spectrophotometric assay. The solubility of 8-OA was greater than 1600 mg/l, a concentration not expected to be exceeded in humans exposed to adenine via adenine fortified blood transfusion.

Human urine was collected from two patients receiving oral adenine treatment for forminotransferase deficiency and urinary DOA and 8-OA solubilities were measured spectrophotometrically. In one patient, a 55-minute post-drug urine collection during intermittent oral adenine dosing (2.12 mg/kg q.i.d.) exhibited DOA and 8-OA concentrations of ~20 and ~60 mg/l, respectively. In another patient, ~100 mg/l DOA and ~40 mg/l 8-OA were measured in a two-hour post-dose (1.4 mg/kg 5x per day) urine sample. In vivo urinary DOA solubilities in these experiments ranged from ~0.5 to ~2.5 times DOA "apparent supersaturability" as predicted from earlier in vitro studies of DOA solubility studies in human urine.

In order to investigate the kinetics of the "apparent supersaturability" of DOA in human urine, timed studies, using our previously established in vitro urine DOA solubility model, were initiated. Preliminary results suggest that the "apparent supersaturability" of DOA in human urine is dependent upon the initial (highest) DOA concentration attained as well as time.

CONCLUSIONS:

The presence of 8-OA does not constitute a serious toxicity of adenine due to 8-OA nephrolithiasis. In vivo solubility of DOA in human urine is much higher than predicted from in vitro solubility studies. "Apparent supersaturation" of DOA is dependent upon magnitude of highest DOA concentration attained and time. These facts emphasize the potential importance of urine flow in minimizing toxicity in patients receiving adenine.

RECOMMENDATIONS:

A pharmacokinetic and metabolic profile of adenine disposition in man, coupled with a knowledge of the kinetics of apparent supersaturability of DOA in human urine, would make a precise quantitative assessment of DOA nephrolithiasis possible. Therefore, the kinetics of "apparent supersaturability" of DOA in human urine should continue to be investigated thoroughly.

PUBLICATIONS:

1. Peck, C. C., F. J. Bailey, G. L. Moore, and T. F. Zuck: Urinary supersaturation by 2,8-dihydroxyadenine (DOA). Transfusion 15:518, 1975 (abstract).

2. Peck, C. C., and L. Z. Benet: A general method for determining thermodynamic pK_a 's of polyprotic, amphoteric compounds from solubility measurements. J Pharm Sci (in press), 1976.

STUDIES 2 and 3

Diffusion of Adenine Into and Out of Red Blood Cells During Cold Storage. Kinetics with Xanthine Oxidase.

Adenine Metabolism and Kinetics in CPD-Adenine Stored Blood. Evaluation of the Phosphate Effect.

PROBLEM:

In order to estimate the magnitude of the potential nephrotoxicity risks of infusing large volumes of stored blood supplemented with adenine, the kinetics of adenine uptake, egress, and metabolism by stored red cells must be known. Data on residual free adenine gained from kinetic studies will be used to make recommendations, if any are required, for limitations on the number of units of blood supplemented with adenine which can be safely transfused acutely to a soldier suffering from hemorrhagic shock. Studies are also required to determine whether changing the concentrations of the other constituents of citrate-phosphate-dextrose (CPD) will alter these kinetics.

RESULTS AND DISCUSSION OF RESULTS:

Studies on adenine uptake when added at time zero to whole blood have been completed. Adenine equilibrates between plasma and red cells in 3 to 30 minutes, depending on temperature (30° to 0°C), and is slowly utilized thereafter during extended blood storage at 4°C .

After 21 days of storage 41% of the adenine remains, and on Day 42 10% of the adenine is still found in the plasma. These studies are now being repeated with packed red cells and additional glucose. Adenine is being studied when adenine and glucose are added on Days 0, 4, or 7. The effects of additional glucose and anticoagulant phosphate concentration are also being evaluated with respect to their influence on the rate of adenine utilization in stored red cells. Initial studies to determine if adenine is transported by passive diffusion or mediated transport were done, but were inconclusive because of limitations in the sampling techniques. The studies need to be repeated with a modified experimental design. Phosphate uptake by red cells yields instantaneous transport of about 10% of the phosphate from CPD into red cells, but little additional uptake is seen during the next 24 hours at 4°C.

A thin layer chromatography separation of adenine and its nucleotides was developed as a screening procedure on PEI-cellulose plates. High pressure liquid chromatography (HPLC) was set up to separate the purines involved in red cell metabolism. This system uses a Pellionex-SCX resin, a 25 μ l of sample, and a complete analysis time of 35 min per sample, which is eightfold faster and 40 times more sensitive than older techniques. A different HPLC procedure, using a Pellionex-SAX resin and an analysis time of about 50 min, was developed to analyze red cell adenine and its nucleotides. Older procedures required a sample 100 times larger and 22 hr per run. Reproducibility of standards in this system was within 0.5% between runs. This analytical tool will be used for completion of adenine metabolism studies and may be used to monitor urine and blood samples from future studies in animal models and humans.

CONCLUSIONS:

Adenine equilibrates rapidly between plasma and red cells, then it is incorporated slowly into the red cell nucleotide pool. The plasma adenine levels fall in an almost linear manner from 8.5 mg/bag on Day 0 to 0.8 mg/bag on Day 42 of 4°C storage. These data were attained in citrate-phosphate-dextrose (CPD), but may be different in acid-citrate-dextrose (ACD) anticoagulant, because of reduced phosphate levels in the latter.

RECOMMENDATIONS:

Additional studies are needed to clarify the kinetics of adenine when it is used in optional additive systems, under packed cell storage conditions, and with varying concentrations of other constituents of CPD.

PUBLICATIONS:

1. Moore, G. L., and M. E. Ledford: A modified fluorometric assay for adenine in plasma and urine. *Biochem Med* 14:147, 1975.
2. Moore, G. L., and M. E. Ledford: The uptake and egress of adenine from human red blood cells. *Transfusion* (in press), 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISB'N INSTR'N	9b. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
75 07 01	D. Change	U	U	NA	NL		
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER		
a. PRIMARY	62772A	3S762772A814			013		
b. XXXXXXXX	62110A	3A162110A821			167		
c. XXXXXXXX	CARDS 114f						
11. TITLE (Precede with Security Classification Code)*							
(U) Effect of Blood-Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
003500 Clinical Medicine, 012900 Physiology, 016200 Stress Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER* Not Applicable				76		.5	
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e. KIND OF AWARD:				77		.5	
f. CUM. AMT.						34	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Div of Combat and Experimental Surg			
				Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME* Neville, J.R., PhD, DAC			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Experimental Hemorrhagic Shock; (U) Trauma; (U) Blood-gas Transport							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The objective is to evaluate the oxygen transport function of blood, particularly oxygen affinity, and define its role in modifying physiologic responses of military personnel to trauma and environmental stress. Related objectives: (a) Continue evaluation of a simple and reliable means for measuring blood-oxygen affinity.</p> <p>(b) Find practical agents for manipulating oxygen affinity, and determine effect of these agents on hemorrhagic shock and hypoxemia in experimental animals.</p> <p>24. (U) Because available techniques for measuring oxygen affinity are generally slow, laborious, and, in retrospect, poorly designed to account for dynamic relationships among red cell nutritional status, metabolic integrity and functional capability, this study will involve a continued effort to improve existing methodology. Using experimental animals, hemorrhagic shock and hypoxemia will be produced under conditions designed to test the effect of altered blood-oxygen affinity on the compensatory responses to these conditions. The pharmacologic manipulation of blood-oxygen affinity will be investigated, in order to discover agents for improving tissue oxygenation.</p> <p>25. (U) 75 07 - 76 09 The biotometry method for determining oxygen affinity of hemoglobin has received further critical evaluation regarding its comparability to <u>in vivo</u> techniques, the effect of temperature and pH, and its general accuracy and precision. Results have compared favorably with conventional but more time-consuming techniques. Using this new methodology, previously unsuspected alterations in the oxygen affinity curve have been detected and these alterations may provide useful insight into problems of tissue oxygenation during battlefield trauma and environmental stress, particularly when associated with hypoxemia. Publications: Neville JR, Clemmer T: Hemoglobin-oxygen affinity in intensive care patients. Fed Proc 34:452, 1975 (abstract).</p>							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery
WORK UNIT NO. 167 Effect of Blood-Oxygen Affinity During
Experimental Hemorrhagic Shock and
Hypoxemia

The in vivo oxygen-affinity of hemoglobin determined in the dog maintained by extracorporeal circulation is identical to results obtained by the biotometry technique. The effects of temperature, pH, carbon dioxide, NaOCN, DPG, hemolysis, erythrocyte age, and species and hematologic differences on blood oxygen affinity obtained with biotometry are substantially identical to those found with independent techniques. Analysis of the heme-heme interaction values obtained with the new technique indicates that, contrary to the widely held belief, this functional attribute is not invariant. Significant differences are observed in apparently healthy individuals as well as during in vivo and in vitro aging of erythrocytes. Low heme-heme interaction is often observed in patients hospitalized for various causes. Analysis of the effect on oxygen transport of these differences has shown that under some circumstances, particularly those producing hypoxemia, altered heme-heme interaction is a more important potential modifier of tissue oxygenation than is altered P_{50} (the index most commonly used to denote change in oxygen affinity). These findings may in part explain why previous efforts to establish the physiologic significance of modified oxygen affinity have been generally inconclusive.

AD-A044 175

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT TECHNICAL REPORT. (U)
SEP 76

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3 OF 5
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BODY OF REPORT

WORK UNIT NO. 167

Effect of Blood-Oxygen Affinity During
Experimental Hemorrhagic Shock and
Hypoxemia

PROBLEM:

In situations that require activation of the organism's compensatory mechanisms to maintain tissue oxygenation, e.g., during work and exercise, exposure to low ambient oxygen pressure, or decreased tissue perfusion resulting from blood loss, it is recognized that some individuals respond more effectively than others to such stress, thus enhancing their performance under these conditions and increasing the likelihood of their recovery from life-threatening trauma. In the military environment, particularly with combat personnel, physical training is utilized in order to increase the effectiveness of these compensatory mechanisms and maximize the probability of surviving hazardous and physically demanding duty assignments. Even under these circumstances, however, fairly wide differences in individual responses to stress and trauma are apparent. It has been demonstrated, for instance, by comparing the work capacity of anemic individuals with subjects having similarly low oxygen capacity produced by carbon monoxide, that the efficiency with which compensatory mechanisms maintain tissue oxygenation is, in part, linked to the oxygen affinity of hemoglobin. Until recently, hemoglobin oxygen affinity, except in certain unusual situations such as with carbon monoxide poisoning and hemoglobinopathies, was considered to be invariant in most individuals, and consequently there was no reason to suspect that this property might contribute to differences in the effectiveness of compensatory mechanisms. The basis of this belief has been weakened, however, by the discovery that hemoglobin oxygen affinity is controlled by the metabolism of the erythrocyte (through production of 2,3-diphosphoglycerate (DPG)) and by reports of wider variations in oxygen affinity, even in supposedly healthy subjects, than had been acknowledged previously. It follows, therefore, that altered oxygen affinity may play a more general role in modifying individual compensatory response mechanisms than has been recognized. For several reasons, proof of this proposition has been difficult to demonstrate. Not only are these responses usually complex, but there have also been limitations in the technical ability to measure oxygen affinity, particularly on a routine basis and in large numbers of subjects. To provide insight into this problem, the present effort has been concerned with the perfection and validation of a technique with which a large enough data base can be accumulated to permit a realistic appraisal

of the possible significance of altered oxygen affinity in both health and disease. It is also desirable to discover how oxygen affinity can be beneficially manipulated, as well as the exact physiologic effects of altered oxygen affinity and the conditions under which these effects are manifested.

RESULTS AND DISCUSSION OF THE RESULTS:

Further validation of the biotometry technique for measuring oxygen affinity has been accomplished by measuring the in vivo oxygen affinity in the dog by an independent technique. Comparison of these results with those obtained with biotometry revealed no meaningful difference between the techniques, even though the biotometry method is considerably faster, demands less technical skill, and requires much smaller quantities of blood. The effect on oxygen affinity of temperature, pH, CO₂, NaOCN, DPG, hemolysis, erythrocyte age, as well as species and hematologic differences have been tested with the technique and found to give results that are substantially the same as those found with independent conventional techniques. Perhaps the most noteworthy of these observations, in that they bear on a possible cause of variation in oxygen affinity between individuals noted in earlier reports, is the finding that young erythrocytes (separated from older cells by centrifugation) have not only a higher P₅₀, but also a greater heme-heme interaction than do older cells. This has been a consistent finding in 12 normal subjects so far tested.

One of the major advantages of the biotometry technique appears to be the precision with which it can detect modification of the shape of the oxygen affinity curve, the so-called heme-heme interaction. Analysis of the variations in the heme-heme interaction that have been obtained in our studies indicates that alterations in this property can contribute significantly to the ability of an individual to maintain tissue oxygenation during stressful situations, particularly those involving hypoxemia. On somewhat inconclusive grounds, the heme-heme interaction previously has been considered to be an invariant property.

CONCLUSIONS:

Uncovering the potential significance of variations in heme-heme interaction may lend substantial insight into the possible basis for individual differences in compensatory responses to stress and trauma. Little research has been reported in this area, and most previous efforts have been concerned only with the position or P₅₀ of the oxygen affinity curve.

With hypoxemia, as may occur at altitude or with pulmonary emboli, changes in P_{50} between 20 and 40 mm Hg have only a small effect on oxygen transport; alteration in the heme-heme interaction under these circumstances, on the other hand, can greatly modify oxygen transport.

RECOMMENDATIONS:

These findings should be verified and extended in acute animal experiments and, eventually, in humans.

PUBLICATIONS:

1. Neville, J. R., and T. Clemmer: Hemoglobin-oxygen affinity in intensive care patients. Fed Proc 34:452, 1975 (abstract).
2. Neville, J. R.: Variation in shape of the hemoglobin oxygen affinity curve and its effect on oxygen delivery. Proceedings of Annual Science Meeting, Aerospace Med. Assoc., San Francisco, California, 1975 (pre-print).
3. Neville, J. R.: Altered heme-heme interaction and tissue oxygen supply: A theoretical analysis. British J Haematol (in press), 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8a. DISSEM INSTR ^a	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62772A		3S762772A814		014	
b. SECONDARY		62110A		3A162110A821		169	
c. THIRDARY		CARDS 114f					
11. TITLE (Precede with Security Classification Code) ^a							
(U) Care of the Combat Injured Eye							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine, 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 05		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		b. FUNDS (In thousands)	
b. NUMBER: ^a Not Applicable				76		.8	
c. TYPE:				FISCAL		.2	
d. AMOUNT:				7T		10	
e. KIND OF AWARD:				CURRENCY			
f. CUM. AMT.				77		.5	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Department of Surgery			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Biggs, Silmon L., LTC, MC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3115			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ^a							
(U) Ocular Trauma; (U) Vitrectomy;							
(U) Traumatic Cataract; (U) Traumatic Vitreopathy; (U) Retinal Detachment							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Injuries involving penetration of the eye often have an unfavorable outcome because of disruption of the normal anatomy of the eye by fibrocellular sheets and bands which develop during the healing process. New instruments have been developed which allow the removal of damaged vitreous, lens and iris. The early debridement of intra-ocular tissues may prevent the loss of many eyes injured in the combat and noncombat military environments.</p> <p>24. (U) A standardized model of penetrating eye injuries will be developed and definitive repair of the corneoscleral laceration, retinal detachment, and aggressive intra-ocular debridement performed at controlled times after injury. Results will be assayed by direct observation, ultrasonographic studies, comparative electroretinography and gross and microscopic anatomic studies. The actual surgery will be performed under the operating microscope and the Machemer vitreous infusion-suction cutter will be used.</p> <p>25. (U) The principal investigator has changed. His studies on chloroquine distribution did not show a time or topographic pattern consistent with the ocular toxicity, suggesting that the functional effects are secondary, possibly to metabolic effects on the ganglion-cell layer. Penicillamine has been shown to both increase cupreous ion removal from the eye and to reduce copper toxicity to the retina. The surgical removal of the iris in nonhuman primates has shown little long-term detrimental effects on the eye, verifying and extending a report in the recent ophthalmic literature. The association of this procedure with further surgical intervention in trauma needs study.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery

WORK UNIT NO. 169 Care of the Combat-Injured Eye

The following investigations have been conducted under this Work Unit:

STUDY NO. 1 Chloroquine Distribution in the Rabbit Eye

STUDY NO. 2 Removal of Copper from the Eye with Penicillamine

STUDY NO. 3 Surgery on Ocular Trauma

Study No. 1 Varying dosages and duration of chloroquine treatment have been evaluated in pigmented and albino rabbits to determine whether the unique geographic and functional changes seen with chloroquine toxicity are a direct result of patterns in chloroquine distribution as had been suggested in the past. Autoradiographic and beta-counting procedures have shown no pattern of chloroquine distribution, either with time or dose, which relates to the unique pattern of chloroquine toxicity. A pilot study in a nonhuman primate indicates that perhaps metabolic effects on the ganglion-cell layer are the most important factors in chloroquine toxicity.

Study No. 2 Penicillamine has been shown to enhance removal of copper ions from the eyes of rabbits. Based upon preliminary electroretinographic data, penicillamine might be a reasonable chemotherapeutic agent in experimental chalcosis.

Study No. 3 The effects of surgical removal of the iris have been evaluated in nonhuman primates. This procedure has been shown to afford excellent surgical visualization. Histopathology and scanning electron microscopy of the corneal endothelium show only transient damage and confirm complete removal of the iris base. Aqueous humor lactate is elevated in the early postoperative period in the aniridic eye but returns to normal by three months. The combination of iris removal with lens removal and partial vitrectomy has been done in a pilot manner and has given excellent results.

BODY OF REPORT

WORK UNIT NO. 169

Care of the Combat-Injured Eye

STUDY NO. 1

Chloroquine Distribution in the Rabbit Eye

PROBLEM:

Chloroquine is a commonly used antimalarial agent and, in large-dose, long-term oral administration, has been shown to cause irreversible ocular damage in humans. To date, the cumulative effects of the administration of low prophylactic doses, followed by significantly higher therapeutic doses in positive cases of malaria or amoebiasis is unknown. Previous studies have indicated that the eye sequesters the chloroquine and retains it for long periods. This study investigated the possibility of differences in distribution of chloroquine in the eye based on either duration of administration or dose of chloroquine.

RESULTS AND DISCUSSION OF THE RESULTS:

Albino rabbits were shown to retain no significant amounts of chloroquine. Pigmented rabbits, on the other hand, retained significant amounts of chloroquine during long periods of administration. Administration times--from one day, one month, one year--were tested, as well as doses from tracer amounts to nearly lethal amounts. Only minor trends were noted, either with time or dose, and a specific distribution pattern of chloroquine damage was not noted either on autoradiography or beta-counting methods.

CONCLUSIONS:

Chloroquine toxicity does not appear to be a result of accumulation of chloroquine within the eye over a long period of time but, rather, due to metabolic damage perhaps first apparent in the ganglion-cell layer.

RECOMMENDATIONS:

None.

PUBLICATIONS

None.

STUDY NO. 2

Removal of Copper from the Eye with
Penicillamine

PROBLEM:

Copper foreign bodies within the eye are frequently encountered following ocular combat trauma. Copper ions are chemotoxic and difficult to remove by surgical means. Penicillamine has been shown to decrease the ocular deposition of copper in Wilson's disease; therefore, its effect on copper removal was investigated.

RESULTS AND DISCUSSION OF THE RESULTS:

Using radioactive cupric nitrate in rabbits, removal of copper from the eye was shown to be enhanced more than sixfold with penicillamine administration over the four-day period of study. This result, combined with previous results showing the decrease in copper chemotoxicity, indicates it to be a potentially useful drug in the treatment of experimental chalcosis (ocular copper toxicity).

CONCLUSIONS:

Penicillamine is a useful chemotherapeutic agent in cupric nitrate induced chalcosis in rabbits.

RECOMMENDATIONS:

The use of penicillamine in the treatment of chalcosis in other animal models deserves investigation.

PUBLICATIONS:

1. Gardner, H. B.: Chemotherapeutics of ocular metallosis. Army Science Conference, 1976.
2. Gardner, H. B.: Effects of deferoxamine on intraocular iron. Experimental Eye Research, in press.

STUDY NO. 3

Surgical Care in Ocular Trauma

PROBLEM:

Ocular injuries in the combat situation are often handled by conservative methods, with hope of ocular rehabilitation after the condition of the patient has stabilized and he is in a CONUS medical center. Therapeutic results have been poor and have not been as satisfactory as the more aggressive approaches currently being employed in civilian

trauma cases. A nonhuman primate model has been used to evaluate removal of the iris to enhance surgical exposure, and thus allowing a more aggressive approach to the care of the combat-injured eye at the secondary case area, which would be the most forward facility for definitive care.

RESULTS AND DISCUSSION OF THE RESULTS:

Iris removal has been readily accomplished in a nonhuman primate. Early postoperative hyphema clears without incidence, and microscopic study shows minimal damage to the eye with a mild loss of corneal endothelial cells in the early postoperative period. A transient hypotony is induced, with rapid recovery in approximately one week postoperatively. An increased aqueous humor lactate level has been found. By three months the sodium, potassium, glucose, and lactate levels in the operated eye had returned to normal, as compared to the fellow control eye. Microscopically and functionally, the drainage system of the eye appears intact with a normal response to pilocarpine. Visualization of the deep ocular structures is greatly enhanced by this procedure, and several animals have been subjected to more extensive ocular surgery with excellent preliminary results. The results have also confirmed a report in the literature which indicates minimal damage from total iris removal.

CONCLUSIONS:

Total iris removal affords excellent visualization permitting more extensive early repair of combat injured eyes.

RECOMMENDATIONS:

The use of surgical aniridia in combination with more extensive ocular surgery should be further investigated in an animal model.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DAOE 6099	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3S762772A814				007	
b. COORDINATING	62110A	3A162110A821				175	
c. COORDINATING							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002600 Biology, 003500 Clin Medicine, 009800 Medical and Hosp Equip, 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07		76 06		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
EXPIRATION:				76		.5	
b. NUMBER: Not Applicable				FISCAL		.1	
c. TYPE:				7T		12	
d. AMOUNT:				YEAR		CURRENT	
e. KIND OF AWARD:				77		.5	
f. CUM. AMT.						39	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				Div of Combat and Experimental Surg			
				Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Neville, J.R., PhD, DAC			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Bensinger, Thomas A., LTC, MC			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Blood Preservation; (U) Temperature; (U) Blood Respiratory Function							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To devise an accelerated assay method for rapid testing of anticoagulant-nutrient preservatives based on observations at elevated temperatures and to determine if rates of change of the observed parameters during storage are predictable on a chemical-kinetic basis. To apply such techniques to the rapid screening of substances and techniques potentially beneficial to the preservation of blood.</p> <p>24. (U) Human blood will be harvested in CPD and CPD-II solutions and stored at approximately 34, 24, 14, and 4°C. Periodic measurement of pH, pO₂, pCO₂, plasma K⁺, oxygen affinity, hematocrit, deformability, 2,3-DPG, ATP, and osmotic fragility will be obtained. Changes of each parameter will be compared directly and on a chemical-kinetic basis.</p> <p>25. (U) 76 04 - 76 09 Data obtained during preservation of blood in CPD solutions and at different temperatures has proved to be amenable to interpretation by thermokinetic theory. Results obtained at high temperatures for ATP, DPG, fragility, pH, and other parameters, require less time and can be used to quantitatively predict the outcome of prolonged low temperature experiments. An important practical implication of these preliminary findings is that moderate decreases in the storage temperature (4°C to 0°C, for instance) can substantially improve storageability of red cells (greater than 50% improvement in levels of both ATP and DPG). Experiments to confirm these predictions are currently in progress.</p>							

*Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68
AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821 Combat Surgery

WORK UNIT NO. 175 Development of a Rapid System for
Assessing Blood Anticoagulant-
Nutrient Preservatives

Observations on whole blood stored at several different temperatures (37, 24, 14 and 4°C) in CPD have shown that the changes occurring in such parameters as adenosine triphosphate (ATP), 2,3 diphosphoglyceric acid (DPG) concentrations, pH, and osmotic fragility are amenable to analysis by thermokinetic laws, an approach that apparently has not previously been used in blood banking technology. Experimental confirmation of this basic objective of the present effort permits two important conclusions: (1) high temperature (and thus rapid) observations of blood storage systems can be used to quantitatively predict the effects of such systems at low temperatures (where considerably longer periods of observation are required); (2) the optimum temperature for liquid whole blood storage is the minimum temperature that can be maintained without freezing.

Our present results indicate that very significant improvements in blood storage can be realized by lowering the storage temperature from 4 to 0°C; preliminary inquiries indicate that this is both technically feasible and economically reasonable.

BODY OF REPORT

WORK UNIT NO. 175

Development of a Rapid System for
Assessing Blood Anticoagulant-
Nutrient Preservatives

PROBLEM:

The objective of this work unit is to devise an accelerated means of evaluating anticoagulant-nutrient preservative solutions (ANPS) used for red cell storage in order: (1) to economize on valuable technical resources; (2) to provide alternative theoretical approaches to understanding the collection and storage lesions produced in preserved red cells; (3) to develop a scientific basis for determining the optimum storage temperature of whole blood. The first objective of this effort was to determine if any of the alterations occurring in stored blood can be predicted on the basis of thermokinetic theory. If such predictions prove valid under experimental conditions, storage systems can be designed to provide to wounded soldiers blood with improved red cell viability and hemoglobin function.

RESULTS AND DISCUSSION OF THE RESULTS:

Blood collected in citrate-phosphate-dextrose (CPD) from healthy donors was divided into four equal portions, each of which were stored at temperatures of either 37, 24, 14, or 4°C. Periodically, aliquots were taken, with care to avoid bacterial contamination, for the determination of pH, PO₂, PCO₂, red cell fragility, DPG, ATP, hematocrit, P₅₀, heme-heme interaction, and oxygen combining capacity. In some instances transmembrane pH was measured. The relative rates with which these parameters changed were derived by determining the time required to either double or halve the original value and were plotted against the reciprocal of the absolute temperature at which the aliquots were stored. In the case of fragility, pH, DPG, and ATP, it was found that such plots yielded straight lines within the limits of error of the technique. Compared to the times required to develop this information at 4°C (approximately five weeks), only two or three days were needed at 37°C.

Some related observations made in the course of these preliminary studies included: (1) the heme-heme interaction of blood from the Black subjects used as donors was significantly lower than that found in Caucasian subjects; (2) the transmembrane pH of red cells stored in CPD at 4°C shows a consistent decrease from approximately 0.2 pH units at 0 days to approximately 0.05 pH units at 20 days and remains constant thereafter; (3) addition of Ca⁺⁺ to heparinized samples of stored blood appears to restore transmembrane pH to normal values.

These preliminary results support the working hypothesis regarding the effect of temperature on blood storage. The predictable nature of the changes occurring in certain parameters during blood storage permits a realistic estimation of the effects to be expected at low temperatures from the much more rapidly occurring changes taking place at high temperatures. A potentially important practical aspect of these results pertain to the logarithmic nature of the relationship between rates of change in the parameter observed and the absolute temperature. An examination of this relationship indicates that fairly large effects on the rate of change is produced by comparatively small variations in temperature. One would predict from the present data that lowering the storage temperature from 4 to 0°C should decrease the rate of change in ATP and DPG, for instance, by 50 to 100%, thus greatly enhancing the quantities of these substances remaining after any given storage period. This simple means of enhancing the preservation of whole blood appears to be both technically and economically feasible, and is unique in providing a common approach for maintaining both ATP and DPG.

CONCLUSIONS:

The use of high temperatures for screening purposes would appear to be a promising means of obtaining preliminary and comparative information on ANPS used for blood banking. Such an approach permits great economy in both time and technical effort, and may accentuate phenomena related to storage lesions that can be observed only with difficulty or not at all during low temperature storage.

The optimum liquid storage temperature for red cells appears to be the minimum temperature that can be used without freezing the cells.

RECOMMENDATIONS:

These preliminary observations should be confirmed, working at temperatures between 0 and 4°C.

In vivo survivability of 0°C stored blood should be tested and compared to 4°C blood.

Equipment permitting storage of blood between approximately -2.0 and +2.0°C should be procured and tested.

PUBLICATIONS:

1. Neville, J. R., T. A. Bensinger, and T. F. Zuck: Effect of temperature on blood storage. Scientific Section, 29th Annual Meeting, American Association of Blood Banks (Preliminary Program), San Francisco, California, November 1976 (abstract accepted for presentation).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6079	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3A762772A813				021	
b. CONTRIBUTING	62758A	3A762758A824		03		078	
c. XEROX 990880 901628	CARDS (114F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Determination of Threshold Data from Coherent and Incoherent Radiation Sources							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
009600 Masers and Lasers 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 12		Cont		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		76	
b. NUMBER: ^a Not Applicable				FISCAL		7T	
c. TYPE:				CURRENT		77	
d. AMOUNT:						3.5	
e. KIND OF AWARD:				f. CUM. AMT.		153	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Department of Biomedical Stress			
				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
RESPONSIBLE INDIVIDUAL				NAME: ^a Beatrice, E. S., LTC, MC			
NAME: Canham, J. E., COL, MC				TELEPHONE: (415) 561-3347			
TELEPHONE: (415) 561-3600				SOCIAL SECURITY ACCOUNT NUMBER:			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Applicable				NAME: Stuck, B. E., DAC			
				NAME: Lund, D. J., DAC		POC: DA	
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Laser Safety; (U) Laser Hazard; (U) Eye Damage; (U) Skin Damage							
23. TECHNICAL OBJECTIVE. ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To characterize by experimentation the biological effects of exposures to military laser systems and to determine exposure thresholds producing damage to eye and skin from these sources.							
24. (U) Threshold data for TEA laser exposures to porcine skin and primate cornea and retinal alterations from neodymium laser radiation will be determined by direct observation and histopathological evaluation of exposed sites. Data from these experiments will be correlated with and incorporated into a biomathematical model with data from exposures to cw carbon dioxide laser experiments for pulse durations from 1 millisecond to 1 second. Additional threshold data utilizing a holmium laser source (106 μ) will further refine the mathematical formulation.							
25. (U) 75 07 - 76 09 The retinal response to noncircular irradiation geometry was determined. Slit-like burn response to slit-like exposure geometry was obtained only for very short exposure durations. No wavelength dependency was seen. Rhesus monkey ocular damage threshold levels were derived for 300 micron retinal image diameter exposures to argon (514.5 μ) radiation at pulse durations of 100 μ sec (ED ₅₀ = 115 μ j), 500 μ sec (ED ₅₀ = 248 μ j), and 1000 μ sec (ED ₅₀ = 354 μ j). Dose response relationships for TEA laser exposures to the cornea and skin have been determined for various response criteria (ED ₅₀ = 515 mj/cm ² - 24 erythema skin; 115 mj/cm ² for a minimal pathologic change and 350 mj/cm ² for minimal corneal opacity).							
TEA = Transversely Excited Atmospheric (laser)							
cw = continuous wave							
j = joule (μ j = microjoule)							
ED = Estimated Dose required to produce a corneal alteration							
μ = micron							
*Available to contractors upon originator's approval.							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A762758A824	Health Effects of Military Lasers
TASK NO.	03	Lasers
WORK UNIT NO.	078	Determination of Threshold Data from Coherent and Incoherent Radiation Sources

The following investigations have been conducted under this Work Unit:

STUDY NO. 1	Skin Effects from TEA Laser Exposures
STUDY NO. 2	Ocular Effects from TEA Laser Exposures
STUDY NO. 4	Threshold Determination of Pulsed and Continuous Wave Neodymium on Primate Retina

The Transversely Excited Atmospheric (TEA) laser operated in the CO₂ mode at 10.6 microns with a pulse duration of 100 nsec at the half power points extending to 250 nsec.

STUDIES NO. 1 and 2. The ED₅₀ radiant exposure required to induce a 24-hour erythema as determined by probit analysis was 515 mj/cm². Partial ablation of the stratum corneum required a dose of 800 mj/cm². The ED₅₀ radiant exposure required to produce corneal opacity observed at 1 hour was 350 mj/cm² for a 2.4 mm irradiance diameter. Subtle corneal changes are being investigated by electron microscopy and cytochemistry. Effects of longer exposure (20 seconds), i.e., near the maximum permissible exposure, are being evaluated.

Study No. 4. The retinal response to noncircular irradiation geometry as a function of exposure duration and wavelength was evaluated. A slit-like burn as a result of slit-like irradiation geometry is a function of exposure duration.

The ocular damage threshold in rhesus monkey eyes has been determined for microsecond pulse duration argon (514.5 nm) laser irradiation when the retinal image diameter is 300 μ at the 1/3 point. The ED₅₀ irradiation levels determined by probit analysis are 115 μ j Total Intraocular Energy (TIE) at 100 μ sec, 248 μ j TIE at 500 μ sec, and 354 μ j TIE at 1000 μ sec.

BODY OF REPORT

WORK UNIT NO.	078	Determination of Threshold Data from Coherent and Incoherent Radiation Sources
STUDY NO.	1	Skin Effects from TEA Laser Exposures

PROBLEM:

Military applications of infrared laser systems are developing at a rapid rate. Although safety procedures and maximum permissible levels have been defined in TB Med 279, there are many exposure situations where safe irradiance levels have been extrapolated and bioeffects data have not been obtained.

The objective of this study is to determine dose-response relationships for various response criteria for Transversely Excited Atmospheric (TEA) laser exposure of skin.

RESULTS AND DISCUSSION OF THE RESULTS:

A TEA laser operating in the CO_2 mode at 10.6 microns was used in this study. The pulse duration at the one-half power points was 100 nsec with the pulse tail extending to 250 nsec. An additional series of exposures were made with nitrogen added to the cavity gas mixture of He and CO_2 extending the pulse tail to 2 μsec . Porcine skin was used in this study. To date, ten pigs have been irradiated with the radiant exposure varying from 10 mj/cm^2 to 3.0 j/cm^2 . A 1 cm irradiance diameter with an intensity distribution that was uniform within 90% of the measured peak irradiance was used for this study. Four exposures per pig for the nine irradiance levels were randomly placed in a 4 x 10 inch grid drawn on a clean clipped pig. The four remaining sites served as control areas. The skin was visually graded immediately post exposure, at 1 hour, and 24 hours. Severity of the observed response varied from minimal erythema observed at 24 hours to an immediate ablation of the entire stratum corneum and partial ablation of the superficial epidermis.

The radiant exposure required to produce an erythemic response, 50% of the time (ED_{50}) as determined by probit analysis was 515 mj/cm^2 . The ED_{50} radiant exposure required to produce any visual response at one hour was 675 mj/cm^2 . The erythemic response was obvious at four hours after exposure. The ED_{50} radiant exposure for immediate ablation of the stratum corneum was 800 mj/cm^2 .

All sections for histopathological evaluation were taken 24 hours after the exposure. The histopathologic effects ranged from eosinophilia of the upper layer of the epidermis at radiant exposures of from 0.15

to 0.25 j/cm^2 . Histopathological analysis after the doses which produced a minimal erythema at 4 to 24 hours showed cellular alterations from $1/3$ to $1/2$ thickness of the epidermis with some dermatitis. Tissue was stained with hematoxylin and eosin.

The difference in radiant exposure required to change the response from a minimal erythema to partial ablation is small. These results indicate the shorter the exposure duration the closer these responses are in levels of radiant energy.

An elongated pulse was created by adding nitrogen (N_2) to the laser cavity. With this pulse, a higher radiant exposure (ED_{50} (approx) 0.775 j/cm^2) is necessary for induction of erythema at 24 hours.

CONCLUSIONS:

The safe radiant exposure for the described exposure conditions given in TB Med 279 is 12.5 mj/cm^2 . Approximately ten times this level was required to produce slight cellular alterations in the superficial epidermis. Consequently, for the response criteria used the present standard appears adequate.

RECOMMENDATIONS:

A comparison of the effects observed for the previous work for longer exposure durations via a damage integral analysis should be made. Cumulative effects from repetitive pulse should be considered because of present military systems operating in the 0-20 Hz range. Effects for pulse durations of 10-15 μsec should be examined in order to correlate the previous 5-10 msec data with this present study at 250 nsec. Depth of alteration as evidenced by histopathology should be correlated with dose.

Study No. 2. Corneal Effects from TEA Laser Exposures.

PROBLEM:

Military laser systems operating in the infrared portion of the spectrum pose a potential hazard to the cornea of the eye. As "safe" levels are developed for permissible human exposure, new research into the responses of the cornea to low levels of infrared radiation and the investigator of the chronic effect of such exposure are required.

RESULTS AND DISCUSSION OF THE RESULTS:

Initial investigations were conducted to determine the threshold dose for the induction of an opacity using the Transversely Excited Atmospheric (TEA) laser operated in the CO_2 mode. Cornea from New Zealand white rabbits and several rhesus monkeys were used in this study.

The intensity distribution was approximately gaussian with a diameter of 2.4 mm at the 1/e intensity points. Three to five exposures were placed in each eye. The response criterion was the biomicroscopic appearance of an opacity one hour after exposure.

Probit analysis of dose response data yielded an ED_{50} level of 350 mJ/cm^2 to produce a minimal opacity in one hour after exposure. Lesions placed at these energy levels involved only the superficial epithelium and were not observed at 24 hours. Radiant exposures of 1.5 to 2.0 J/cm^2 caused an explosive ablation upon impact and the immediate lesion diameter of 2.7 mm. Perforation of the epithelium, as evidenced by fluorescein staining, was observed at these high exposure levels. Near threshold lesion diameters were between 0.7 to 0.9 mm. The corneas were excised at 24 hours after exposure and the endothelium was stained with trypan blue. Preliminary results with trypan blue are inconclusive and require further investigation. Endothelial alterations resulting from the thermal as well as mechanical insult for these short deposition times may result in chronic corneal deterioration which is not observed at the usual 1-hour, 24-hours, and 1-week observation periods. Determination of the spot size dependence of the TEA laser corneal exposure has been started and statistics are being collected.

Two rhesus monkey corneas were exposed to 200 mW/cm^2 which is two times the present maximum permissible exposure at 10.6 microns. The entire cornea was exposed with a nearly uniform intensity distribution. Although premature drying of the corneal tear film was observed 15 sec into the exposure at 200 mW/cm^2 , no observed opacity or change in corneal curvature resulted. Under light microscopy, no histopathological alterations were observed; however, under electron microscopy, inconclusive changes in the corneal endothelium were noted in the exposed eye as compared to the control eye. Further evaluation of this finding is required.

Determination of the dose-response relationship for the holmium laser at 2.06 microns have been delayed because of a defect in the holmium rod. (Studies will be reinstituted when a new rod, without defects, arrives). Several exposures were made in one rhesus monkey cornea. Because of the defect in the holmium rod, useful dosimetry and exposures were not possible; however, 100 mJ in a beam diameter of 2.5 to 3.0 mm produced an above threshold corneal opacity. Several corneal tissue samples were prepared for the histocytochemical analysis by Dr. K.C. Tsou under AMRDC contract number DAMD-17-74-C-4143. The dose for these evaluations was from 3.5 W/cm^2 to 35 W/cm^2 for a 100 msec exposure at 10.6 microns.

CONCLUSIONS:

It appears from these data that present levels for CO₂ laser exposures produce no significant corneal alteration in the acute phase for the criteria used in present experiments.

RECOMMENDATIONS:

Corneal endothelium and its susceptibility to laser injury must be evaluated. Histopathological studies should be performed on specimens stained with trypan blue. Imbedded corneas should be evaluated with electron microscopy. In vivo examinations should be made with a specular microscope. Overall chronic complications resulting from endothelial alteration may be more significant than corneal epithelial changes. Unpublished data by Ham, et al., indicate that fluorescence staining within the cornea was diffuse 48 hours after exposure when corneas were exposed to near the maximum permissible dose for a 1 nsec CO₂ laser pulse. Although data reported are an order of magnitude higher than this value for a 100 nsec pulse duration, evaluation of fluorescence staining 48 hours after exposure may result in a more sensitive measure of alteration.

Study No. 4. Threshold Determination of Pulse and Continuous Wave Neodymium on Primate Retina.

PROBLEM:

A need to determine the retinal response to noncircular irradiation geometry has been generated by the anomalous results obtained with the gallium arsenide (GaAs) laser in previous experiments in which the retinal response to a slit geometry retinal exposure was always circular or annular.

Ocular damage threshold data were required for large diameter microsecond-pulse-duration retinal irradiation in support of theoretical analysis of retinal damage mechanisms.

Ocular damage threshold data in the near infrared (IR) as a function of pulse duration and repetition rate are required in support of Project MILES which is currently restricted by existing laser safety regulations.

RESULTS AND DISCUSSION OF RESULTS:

Comparative experiments have been conducted on the retinal response to noncircular retinal irradiation geometry. In this experiment continuous wave neodymium and Q-switched neodymium lasers (1.06 μ), as well as Q-switched ruby (0.6943 μ) and continuous wave argon (0.5145 μ) lasers were used with a common delivery system to produce slit geometry retinal irradiance in rhesus monkey eyes. The exposure sites were evaluated ophthalmoscopically for appearance and shape of the resulting damage.

Q-switched lasers, both ruby and neodymium, produced elongated lesions of the same shape as the irradiation geometry. Continuous wave neodymium and argon lasers produced round burn responses to slit-like retinal irradiation for all exposure durations longer than 12 msec. These results are consistent with thermal models of retinal damage.

An acousto-optical modulator was procured for use as a fast shutter for production of microsecond duration pulses. This shutter was installed in the argon laser delivery system prior to use with the continuous wave neodymium. This shutter was used to determine damage thresholds in the rhesus monkey eye for exposure durations of 100 sec, 500 sec and 1000 sec to 514.5 nm argon radiation. The retinal irradiation area was 300 microns diameter at the 1/e intensity point. ED_{50} levels obtained by probit analysis were 115 j total intraocular energy (TIE) at 100 sec, 248 j TIE at 500 sec, and 354 j TIE at 1000 sec.

In support of Project MILES, procurement action has been initiated for an acousto-optic Q-switch for the continuous wave neodymium laser and for an erbium laser system. In addition, a technique has been developed and implemented for evaluation of the beam characteristics of a prototype GaAs MILES laser device.

CONCLUSIONS:

The ability of the retina to produce a noncircular burn response to a noncircular irradiation geometry is exposure duration dependent, but not wavelength dependent.

Insufficient data for small irradiation diameter microsecond-pulse-duration argon retinal irradiation exist to allow meaningful comparison with the large spot diameter data in support of theory.

RECOMMENDATIONS:

Further ocular damage threshold data for microsecond-pulse-duration exposures are required both in the visible and near IR regions. Cumulative effects from repetitive pulses must be evaluated as a function of interpulse spacing and number of pulses.

PUBLICATIONS:

1. Beatrice, E.S., H. Zwick, and D.J. Lund. Bioeffects research in the determination of laser hazards. NATO/AGARD 33rd Aerospace Medical Panel Meeting, September 1976.
2. Beatrice, E.S. and D.J. Lund. Characteristics of damage produced by noncircular retinal laser irradiation. LAIR Report, July 1976.

3. Lund, D.J. Evaluation of beam characteristics of multiple integrated laser engagement simulator (MILES) laser device. LAIR Report, July 1976.

4. Peterson, L.J. and D.J. Lund. Spectrophotometry of the canine bladder. LAMC Report, July 1976.

5. Beatrice, E.S. and D.J. Lund. Retinal thresholds for microsecond argon laser exposures. LAIR Report, September 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OB 6790	76 09 30	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62759A	3A762759A831			001		
b. SECONDARY							
c. THIRDARY	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code) ^a (U) Delayed Type Skin Reaction and Lymphocyte Transformation in Cutaneous Diseases (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 - Clinical Medicine; 010100 - Immunology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
67 04		76 09		DA		C. In-House	
17. CONTRACT / GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER:				76		3.0	
c. TYPE: Not Applicable				77		8	
d. KIND OF AWARD:				CURRENT		15	
e. CUM. AMT.				77		0	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Dermatology Research			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Kerbs, Sharon, Ph.D, DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5455			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Greenberg, J.H., LTC, MC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Lymphocyte Transformation (LT); (U) Delayed Hyper-sensitivity; Leukocytes; (U) Chemotaxis (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To determine if environmental conditions or drug therapy can effect cutaneous dermatophytosis. To investigate methods of inducing immunity. To study whether an animal with "activated" macrophages can curtail fungal infections more efficiently than an animal with "non-activated" macrophages. To obtain a chronically infected guinea pig.							
24. (U) Lesions on infected guinea pigs are exposed to wet occlusion, antifungal drugs, or steroids and lesion development is compared to untreated control lesions. Development of protective immunity is determined following immunization by injection of fungal antigens or following removal of spore-seeded sites before lesions develop. The influence of "activated" macrophages on curtailing infections is studied by initiating infections on top of skin areas containing "activated" macrophages or on top of control areas. We attempted to obtain chronically infected guinea pigs by injecting newborns with soluble antigens.							
25. (U) 75 07 - 76 09. Continual wet occlusion from the time of spore inoculation reduces lesion size and promotes healing. Oral griseofulvin reduces inflammation, lesion size, and prevents alopecia. Topical steroid treatment was ineffective. This work unit is being terminated.							

^a Available to contractors upon originator's approval.DD FORM 1498
1 MAR 66PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65
AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762759A831 Other Tropical Medicine
WORK UNIT NO. 001 Delayed Type Skin Reaction
and Lymphocyte Transformation
in Cutaneous Diseases

The following investigations have been conducted under this work unit:

- STUDY NO. 2 Immunization Against Dermatophytes
- STUDY NO. 4 Attempts to Induce Tolerance to Trichophytin
- STUDY NO. 5 Effect of Inoculating Spores on top of an Area of Skin Already Manifesting a Delayed Type Hypersensitivity Reaction.
- STUDY NO. 6 Effect of Wet Occlusion and Drug Therapy on Course of Fungal Infections.
- STUDY NO. 7 Effect of Drug and Steroid Therapy on Cutaneous Fungal Infections.

Study No. 2 Partial protective immunity to dermatophytosis can be demonstrated following infection of lyophilized hyphae in complete Freund's adjuvant in guinea pigs. Partial protective immunity was also demonstrated in animals which were inoculated on the skin with live spores and the inoculum removed before a lesion developed. This may be a new method of inducing protective immunity.

Study No. 4 Attempts to induce tolerance to trichophytin in newborn guinea pigs has been unsuccessful. Animals become sensitized instead of tolerant.

Study No. 5 The presence of a delayed type hypersensitivity response at the site of spore inoculation does not alter a primary infection, however, it does alter a secondary infection which may argue for a role of antibodies in fungal infections.

Study No. 6 Continual wet occlusion from the time of spore inoculation will reduce the subsequent lesion size and quicken healing. Continual wet occlusion in conjunction with oral griseofulvin is more effective than either treatment alone.

Study No. 7 The size of a fungal lesion can be reduced by topical application of miconazole nitrate (Monostat) but not by topical steroid treatment.

BODY OF REPORT

WORK UNIT NO. 001

Delayed Type Skin Reaction and
Lymphocyte Transformation in
Cutaneous Diseases

STUDY NO. 2

Immunization Against Dermatophytes

PROBLEM:

A vaccine preventing dermatophytosis would be beneficial to the Army. Complete immunity may be difficult to achieve because experimental infection results in only partial protective immunity (smaller lesions of shorter duration). More fungal spores are required to reinfect man than are needed to induce an initial experimental infection.

EXPERIMENT NO. 1

Injection of Antigens

RESULTS AND DISCUSSION OF THE RESULTS:

Guinea pigs were injected with (a) complete Freund's adjuvant (CFA) alone, (b) CFA mixed with culture fluid that fungi had grown in, or (c) CFA mixed with killed lyophilized hyphae and culture fluid. Lesions that developed on control animals were larger in size (689mm^2) than lesions which developed in those injected with culture fluid (259mm^2), or culture fluid plus hyphae (216mm^2). These smaller lesions are comparable in size to normal secondary lesions.

Results indicate that injection of either culture supernatant or hyphae can immunize a guinea pig and give partial protective immunity comparable to that gained by a primary experimental infection.

CONCLUSION:

Injection of either culture supernatant or killed lyophilized hyphae in CFA can induce partial protective immunity.

RECOMMENDATION:

An injection procedure which will result in total protective immunity without the need for complete Freund's adjuvant needs to be found.

PUBLICATIONS:

None.

RESULTS AND DISCUSSION OF RESULTS:

Guinea pigs were infected with spores. The spore inoculum was physically removed at daily intervals thereafter. Animals that had the inoculum removed during the first 2 days were skin test negative. Subsequently when they were infected, lesions corresponded to primary infections. Animals that had the inoculum removed after 3 to 5 days were skin test positive to trichophytin and demonstrated partial protective immunity. Lesions in control animals did not develop until Day 7. Therefore, animals have partial protective immunity without having had an active lesion.

CONCLUSION:

Partial protective immunity, comparable to that gained by an active primary infection, can be acquired by removing the inoculating fungi before a lesion has time to develop.

RECOMMENDATIONS:

Allowing fungi to proliferate for a few days to effect immunization and then removing the fungi before a lesion is formed may be a new way to induce immunity. Further investigation of methods to eliminate the fungi before lesions develop are necessary.

PUBLICATIONS:

None.

STUDY NO. 4

Attempts to Induce Tolerance to
Trichophytin Antigen

PROBLEM:

An immunologically unresponsive animal (tolerant) to trichophytin may have prolonged or chronic infections. A chronically infected animal would be useful to the Army for studies involving treatment.

RESULTS AND DISCUSSION OF THE RESULTS:

New born guinea pigs (less than 24 hours) were injected in their footpads with soluble trichophytin antigens. Controls consisted of adult animals given similiar injections. After 14 days, animals were skin tested with trichophytin antigen. Newborn as well as adult guinea pigs were skin test positive. The subsequent dermatophytosis was similiar in both groups and did not become chronic in newborns.

CONCLUSION:

Tolerance to trichophytin antigen was not induced in newborn guinea pigs by injection of soluble antigens.

RECOMMENDATIONS:

Other methods of inducing tolerance should be attempted.

PUBLICATIONS:

None.

STUDY NO. 5

Effect of Inoculating Spores on
Top of an Area of Skin already
Manifesting a Delayed Type Hyper-
sensitivity Reaction.

PROBLEM:

The normal mechanism in fungal infections that induces healing is not understood. We investigated the effect of activated macrophages on curtailment of fungal infections.

RESULTS AND DISCUSSION OF RESULTS:

Virgin animals were infected at two sites. One was a control site and the other was a site demonstrating delayed type hypersensitivity to dinitrochlorobenzene. In animals where this was their primary infection, the course of the infection was similar at both sites. This indicated that the presence of activated macrophages did not curtail the infection. In experienced animals that already had partial protective immunity, the presence of the delayed type hypersensitivity response further reduced the lesion size. These results do not clarify what determines the lesion size in experienced animals. One possibility could be humoral antibodies interacting with lymphocytes.

CONCLUSIONS:

A pre-existing delayed type hypersensitivity response influences the lesion in an animal undergoing a secondary infection but does not influence lesion development in animals undergoing primary infections.

RECOMMENDATIONS:

The interaction of antibodies and cellular responses should be investigated.

PUBLICATIONS:

Kerbs, S., K. Jesrani and J. Greenberg: Effect of pre-existing inflammation on the course of dermatophyte infections. (Abstract) American Society of Microbiology, 1976.

STUDY NO. 6

Effect of Wet Occlusion and Drug
Therapy on Course of Fungal Infections

PROBLEM:

Environmental conditions are known to affect the prevalence of dermatophytosis. These investigations study the effect of continual wet occlusion and the efficacy of oral griseofulvin under these conditions. This information is relevant to the treatment of soldiers.

RESULTS AND DISCUSSION OF THE RESULTS:

The effect of occlusion on the duration and severity of acute dermatophytosis was assessed in guinea pigs given standard Trichophyton mentagrophytes infections. After inducing infections by occluding spore-seeded sites for 4 days, occlusive patches were left in place in some animals and permanently removed in others. Some animals in each group were treated with oral griseofulvin and some were given no drug treatment. Lesions were smallest in animals treated with both occlusion and griseofulvin, largest in untreated controls, and of intermediate size in those treated with either occlusion or griseofulvin alone. The degree of inflammation was no different in animals treated with continuous occlusion alone than in untreated controls, but was markedly reduced in both groups given griseofulvin. Occlusion did not prevent the development of alopecia, but administration of griseofulvin did prevent alopecia. Lesions healed significantly more rapidly in animals treated with occlusion and griseofulvin than in animals treated with griseofulvin alone.

CONCLUSION:

These results suggest that continuous occlusion of the inoculated site can have a marked effect on the course of acute dermatophytosis.

RECOMMENDATIONS:

The possibility of wet occlusion as an adjunct to drug therapy should be further investigated.

PUBLICATIONS:

None.

STUDY NO. 7

Effect of Drug and Steroid
Therapy on Cutaneous Fungal
Infections

PROBLEM:

Treatment of dermatophytosis is an important problem, for the military. The effect of steroids on fungal lesions is uncertain.

RESULTS AND DISCUSSION OF RESULTS:

Miconazole nitrate will reduce fungal lesion size in guinea pigs, but topical steroid cream appears ineffective. Topical steroid treatment did not produce chronic infections.

CONCLUSIONS:

Topical steroids did not influence dermatophyte infections in guinea pigs.

RECOMMENDATIONS:

Systemic steroid therapy may be more effective in producing a prolonged infection, but studies should be done to prove (or disprove) its efficacy.

This work unit is being terminated. A portion of these studies will continue under a new Work Unit.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6073	76 09 30	DD-DR&E(AR)636	
3. DATE PREV. SUMM ^a	4. KIND OF SUMMARY	5. SUMMARY SCT ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA CONTRACTOR ACCESS	9b. LEVEL OF SUM
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62759A	3A762759A831	00	002			
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Serodiagnosis of American Leishmaniasis							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
0010100 Microbiology; 002600 Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 11		76 09		DA		C. In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
EXPIRATION:				76		1.5	
b. NUMBER * Not Applicable				FISCAL 7T		.4	
c. TYPE				CURRENT		12	
d. AMOUNT:				77		0	
e. KIND OF AWARD:				0		0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME * Letterman Army Institute of Research				NAME * Letterman Army Institute of Research			
ADDRESS * Presidio of San Francisco, CA 94129				ADDRESS * Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME * Childs, G.E., CPT, MS			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3564			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Moussa, M.A., LTC, MS			
				NAME: Rutledge, L.C., DAC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Leishmania; (U) Serology; (U) Immunology; (U) Antigen Fractionation; (U) Epidemiology; (U) Diagnosis; (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) American leishmaniasis, which is caused by protozoan parasites of the genus <u>Leishmania</u>, is characterized by cutaneous granulomas and mild to serious ulcerative destruction of the nose, mouth, and pharynx. Diagnosis is not generally possible until development of the lesions. The purpose of this investigation is to develop a simple and more sensitive serological test for early diagnosis of American leishmaniasis among military personnel serving in endemic areas.</p> <p>24. (U) A <u>Leishmania</u> tissue culture system suitable for antigen production will be developed. <u>Anastigote</u> antigens will be purified for use in standard serological tests. Column chromatography fractionation procedures will be used to isolate and purify the antigens. Specific antisera will be prepared in rabbits using the purified antigens. Sensitivity of antigen will be tested using immune and control sera from patients with American leishmaniasis. Selectivity will be tested against closely related species as well as against American trypanosomiasis. Field tests will be conducted to determine suitability for diagnostic and epidemiological application.</p> <p>25. (U) 75 07 - 76 09 Methods have been developed and optimized for the isolation and purification of amastigotes of several leishmanial strains by trypsinization, and for the transformation of promastigotes to amastigotes by temperature elevation. The use of insect tissue culture media has made it possible to culture and harvest large quantities of promastigotes for antigen preparations. Leishmanial antigens have been adapted to standard serological procedures to demonstrate leishmanial antibodies in sera from infected experimental animals. See Childs, G.E., McRoberts, M.J. and Foster, K.A.: Partial purification of amastigotes from cutaneous lesions of American leishmaniasis. J. Parasitol. 1976 (in press).</p>							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

209

U.S. GPO: 1974-540-843/8691

ABSTRACT

PROJECT NO. 3A762759A831

Other Tropical Medicine

WORK UNIT NO. 002

Serodiagnosis of American
Leishmaniasis

Methods previously developed in this laboratory for the propagation and collection of leishmanial parasites have been refined and evaluated. These techniques were extended to additional strains of leishmanial parasites for the production of adequate yields of antigen for use in serological procedures. Trypsinization and primary tissue culture techniques proved highly suitable for many pathogenic strains for providing large yields of partially purified amastigotes and for inducing infections in animals in 1 to 3 weeks instead of 8 to 10 months. Conditions suitable for the transformation of promastigote to amastigote-like forms by elevated temperatures have been determined for several strains. L. mexicana (OCR-B) was unique in responding to this technique. Nearly 100% transformation with a 20-fold increase in yield was achieved following elevation of incubation temperature from 24 to 35C. Several insect tissue culture media were found to be suitable for large scale production of promastigotes of several strains of L. brasiliensis, L. mexicana, and L. enriettii. Up to 16 ml of packed promastigotes of L. mexicana (OCR-B) have been collected from 5 liters of Schneider's Medium. Leishmania antigens were prepared and adapted to standard serological tests including: complement fixation, passive hemagglutination, and indirect fluorescent antibody for the detection of leishmanial antibodies in experimental animal sera. Serum antibodies were detected in immunized rabbits and infected hamsters and guinea pigs with homologous antigen. The feasibility of solid phase radioimmunoassay system for the detection of leishmanial antibodies has also been determined. Information derived from this study will assist in the development of a simple but effective serological test for use in detecting early infections among military personnel serving in, or returning from, endemic areas.

BODY OF REPORT

WORK UNIT NO. 002

Serodiagnosis of American
Leishmaniasis

PROBLEM:

Serological techniques for the detection of leishmanial infections lack specificity and the sensitivity to detect low levels of circulating antibodies. Thus, infections are not ordinarily diagnosed until a sizeable lesion develops. Early diagnosis would permit early treatment with less risk of serious complications from mucocutaneous or visceral involvement. This study is designed to develop a simple but effective serological test for use in detecting early infections among military personnel serving in, or returning from, endemic areas.

RESULTS AND DISCUSSION OF THE RESULTS:

The development of methods for the isolation and propagation of amastigotes and promastigotes was continued and extended to additional strains of Leishmania brasiliensis, L. mexicana, and L. enriettii. Trypsinization and primary tissue culture techniques for isolation of amastigotes were extended to several strains. Large numbers of amastigotes can be collected from experimental animals for antigen preparation. Amastigote yields approaching 3×10^7 for L. brasiliensis, 1×10^7 for L. mexicana (OCR-B), 7×10^8 for L. mexicana amazonensis, and 5×10^7 for L. enriettii were achieved. This procedure has made it possible, for the first time, to quantitate and standardize leishmanial infections in animals and to eliminate contaminating organisms from the inocula. The effect of purification on the incubation time of infection is also significant. Purified amastigotes of L. brasiliensis and L. mexicana produced lesions in 1 to 3 weeks in contrast to 6 to 8 months as reported in the literature for these strains.

Conditions suitable for the transformation of promastigote to amastigote-like forms by elevated temperature have been determined for several strains. Transformation rates of 94 to 100% were achieved for all strains when the incubation temperature was increased from 27C to 37C with a marked decline in total number of parasites by Day 4. At lower temperatures (24C to 35C) transformation occurred at a rate of only 4 to 34% and there was a general increase in total number of parasites over a 4-day period. L. mexicana (OCR-B), which showed a 96% transformation rate with a 20-fold increase in total number of parasites, was exceptional. Ultrastructural examination of the transformed parasites revealed morphological characteristics similar to amastigotes observed in vivo by other investigators. Although there was evidence of degenerating parasites, most appeared intact and viable.

Several media were evaluated for optimal growth of six leishmanial

strains on the basis of maximum parasite yields, generation time, and purity of parasite collection. Three strains of L. mexicana, two of L. brasiliensis, and L. enriettii were grown in rabbit blood-based media (Senekje's, NNN (Novy, MacNeal, Nicolle), Tanabe's), insect tissue culture media with 5 to 20% fetal calf serum (FCS) (Grace's, Schneider's, and Mitsuhashi's), and in vitro cell culture systems (LLC-MK₂ in Minimal Essential Medium (MEM), Vero in MEM, and Aedes in Mitsuhashi's). Maximum yield of parasites in in vitro cell culture systems was generally greater than in blood-based media. However, the usefulness of these in vitro cell culture systems is limited since they are not axenic and present additional difficulties in isolating the parasites which develop intracellularly.

The insect tissue culture media were highly suitable for large batch cultivation of promastigotes. Up to 16 ml of packed promastigotes of L. mexicana (OCR-B) were recovered from 5 liters of Schneider's Medium with 15% FCS following incubation for 5 days at 27 C with aeration. The three insect cell culture media were superior to either the blood-based media or the cell culture systems in a number of respects. Insect cell culture media are axenic and monophasic so that collection of relatively pure promastigotes required only centrifugation. These media provided accelerated growth and high yields of promastigotes. Yields of L. brasiliensis and L. mexicana approached or exceeded 1×10^8 promastigotes/ml from blood-based media. The growth phase of the parasites in the insect cell culture media occurred early and maximum yields were attained by Day 5 to 7. The death phase was rapid; within two days the numbers decreased to 1×10^4 .

Insect tissue culture media prepared in our laboratory from basic ingredients were superior to the commercial products. The prepared media consistently produced increased parasite yields with a substantial cost reduction.

Titers of 1:32,000 by complement fixation (CF) were demonstrated in rabbits following immunization with whole or solubilized promastigote antigens, while chickens and S-180 sarcomas in mice did not show a response. Titers of 1:64 and 1:128 by CF were detected in sera of hamsters and guinea pigs recovered from infection with L. mexicana amazonensis and L. enriettii, respectively. Limited trials with soluble L. mexicana promastigote antigen and rabbit anti-L. mexicana sera in solid phase radioimmunoassay have shown that this method can also be used to demonstrate leishmanial antibody.

CONCLUSIONS:

Methods have been developed and optimized for the isolation and purification of amastigotes of several leishmanial strains by trypsinization and for the transformation of promastigotes to amastigotes by temperature elevation. The use of insect tissue culture media has made it possible to culture and harvest large quantities of promastigotes for antigen preparation. Leishmanial antigens have

been adapted to standard serological procedures to demonstrate leishmanial antibodies in sera from infected experimental animal models.

RECOMMENDATIONS:

Series of antigens should be isolated and purified from promastigotes and amastigotes by use of column chromatography and electrofocusing techniques. The antigens should be tested in a variety of standard in vitro serological procedures for the detection of low-level circulating antibodies. Because of the increased sensitivity, solid phase radioimmunoassay should be standardized and evaluated for use as a routine diagnostic system for cutaneous leishmaniasis. Studies to characterize the nature of temperature-induced amastigotes and to evaluate their potential use as antigens should be expanded.

NOTE: Studies formerly conducted under this work unit will continue under a new work unit 015, entitled "Diagnosis and Prevention of American Leishmaniasis in Military Personnel," Project 3M762772A810, Military Skin Disease.

PUBLICATIONS:

Childs, G.E., M.J. McRoberts and K.A. Foster. Partial purification of amastigotes from cutaneous lesions of American leishmaniasis. J. Parasitol. 1976. (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6085	76 09 30	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		62759A		3A762759A831		00	
B. CONTRIBUTING						003	
C. CONTRIBUTING		CARDS 114 (F)					
11. TITLE (Precede with Security Classification Code) ^a							
(U) Ecology and Control of Sylvatic Plague at Fort Hunter Liggett (FHL)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002600 Biology; 005900 Environmental biology; 010100 Microbiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 01		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
B. NUMBER ^a Not Applicable				76		1.5	
C. TYPE:				FISCAL		7T	
D. AMOUNT:				CURRENT		4	
E. KIND OF AWARD:				77		0	
F. CUM. AMT.						0	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a Presidio of San Francisco, CA 94129				ADDRESS ^a Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME ^a Moussa, Moufied A., LTC, MS			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Cavanaugh, D.C., COL, MS (WRAIR)			
				NAME: Rutledge, L.C. & Williams, J.E., CPT			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Plague; (U) Ecology; (U) Flea; (U) Rodent; (U) Serology; (U) Insecticide; (U) Surveillance; (U) Zoonoses Control							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To evaluate the health hazard of plague at Fort Hunter Liggett (FHL) and to determine the most feasible method of plague control in order to minimize the risk of troop exposure.</p> <p>24. (U) Wild rodents will be trapped at FHL; their population fluctuations and flea indices will be monitored. Blood samples will be obtained from captured animals for testing of sera for indirect hemagglutinating antibody using Fraction I (FI) antigen. Flea pools and frozen tissues will be shipped to WRAIR for isolation of plague bacillus. Captured rodents will also be tested for susceptibility to plague infection at WRAIR. Susceptibility of vector fleas to insecticides will be determined. Field evaluation of the oral systemic insecticides, Trichlorfon and Phoxim for control of fleas on wild rodents will also be attempted.</p> <p>25. (U) 75 07 - 76 09 Baseline data on the seasonal incidence of wild rodents and their associated fleas have been completed. Serological evidence of plague infection was detected among wild carnivores, dogs, cats and a squirrel captured at FHL. Phoxim was confirmed to be superior to Trichlorfon in control of fleas on squirrels. Application of the anticoagulant rodenticide diphacinone in oat groat formulations achieved nearly complete control of squirrels. Long term control will require the continuous application of phoxim and diphacinone treated bait.</p>							

ABSTRACT

PROJECT NO. 3A762759A831

Other Tropical Medicine

WORK UNIT NO. 003

Ecology and Control of
Sylvatic Plague at Fort Hunter
Liggett

Baseline data on the seasonal incidence of wild rodents and their associated fleas have been completed. Serological evidence of plague infection has been detected in animals captured at Fort Hunter Liggett. Thus far, 14 sera taken from wild carnivores, dogs, cats and a squirrel have been positive for plague. The evaluation of oral systemic insecticides for control of fleas on rodents has shown that phoxim is superior to trichlorfon. The two major flea vectors associated with squirrels were found susceptible to selected organo-phosphate/chlorine insecticides and carbamates. The commercial formulation of the anti-coagulant rodenticide, Ramik Green(R) was more effective in causing mortality of squirrels in the laboratory than oat groat formulations of diphacinone or universal concentrate. In the field, diphacinone combined with phoxim resulted in nearly complete control of ground squirrels in 2 weeks. A substantial rise of fleas was observed on the surviving animals in the rodenticide treated plots, particularly in the absence of insecticide treatment. Removal of the treated bait resulted in squirrels becoming re-established indicating a need for continuous application in order to maintain effective control. Information obtained from these studies will assist in the evaluation of the health hazard at Fort Hunter Liggett, and in determining the most feasible method for plague control in order to minimize the risk of troop exposure.

BODY OF REPORT

WORK UNIT NO. 003

Ecology and Control of
Sylvatic Plague at Fort
Hunter Liggett

PROBLEM:

A sylvatic plague hazard to humans is of current concern at Fort Hunter Liggett in California. This installation is used by units of the Combat Development and Experimentation Command, and other units for maneuver and combat training.

The ground squirrel, which proliferates on Fort Hunter Liggett, is one of the most important sources of sylvatic plague infections of humans in California. Populations of this rodent were kept in check with toxicants until prohibited by an Executive Order banning the use of certain poisons for rodent control on federal lands. Subsequently, considerable increase in the densities of these rodents and their flea ectoparasites became apparent. Since high densities of susceptible rodents in plague endemic regions invite epizootics, it is feared that this crowding condition may become important in activating plague outbreaks. This problem prompted Headquarters, MEDDAC at Fort Ord, California to request assistance from USAMRDC in evaluating the health hazard of plague and in determining the most feasible method of plague vector control at Fort Hunter Liggett.

RESULTS AND DISCUSSION OF THE RESULTS:

Plague surveillance continued at Fort Hunter Liggett in an effort to delineate the factors involved in maintaining enzootic foci on that installation. To date, a total of 1928 animals have been trapped, from which 57,316 fleas and 1139 serum samples have been collected. The California ground squirrel continues to be the most abundant species representing nearly 90% of the captured animals. Seven species of carnivores (bobcat, coyote, dog, grey fox, house cat, mountain lion, and striped skunk) were collected. Small rodents collected were the brush mouse, deer mouse, California vole, California pocket mouse, desert wood rat, desert cottontail, and kangaroo rat. The squirrel population estimates ranged up to fifty animals per acre. The population remained at relatively high levels throughout the summer and early fall and declined moderately during the winter months.

Nine species of fleas were collected from the captured animals. Of the three species associated with ground squirrels, Diamanus montanus and Hoplopsyllus anomalus were most abundant. These two species have been previously implicated in the transmission of plague in the United States. D. montanus was predominant in winter and early spring while H. anomalus was most abundant during the summer. The monthly average number of fleas per squirrel ranged from 20 in April to 70 in June and July.

Attempts to detect evidence of plague foci on the installation have revealed the presence of plague infection among animals. Of the 1029 sera tested so far, 14 samples taken from wild carnivores, dogs, cats and a squirrel have been positive for plague when tested by indirect hemagglutinating antibody using Fraction 1 antigen. A total of 31,000 fleas processed for plague bacterium isolation proved negative. Also, 136 rodent carcasses were processed, but isolations of Yersinia pestis were not obtained.

Two oral systemic insecticides, phoxim and trichlorfon were evaluated as potential candidates for flea control. Phoxim proved highly effective. This material reduced fleas on the squirrels by nearly 80% within 6 days, and by more than 95% within 12 days of initial treatment. The two main flea vectors were also tested for their susceptibility to malathion, diazinon, carbaryl, and DDT. D. montanus appeared less susceptible to these insecticides than H. anomalus.

Selected anticoagulant formulations were evaluated in the laboratory for their rodenticidal efficacy against the California ground squirrel prior to field testing. These included 0.005% and 0.01% diphacinone (2-diphenylacetyl-1,3-indandione) in oat groats; a commercial preparation, Ramik Green^(R), containing 0.005% diphacinone; and the standard military rodenticide, universal concentrate, (A.I. 0.05% 2-isovaleryl-1,3-indandione) in oat groats. Ramik Green^(R) was most effective in the control of squirrels. Animal mortality occurred at an average of 12, 14, 13, and 15 days for Ramik Green^(R), diphacinone 0.005% and 0.01%, and universal concentrate, respectively. In general, the squirrels consumed only 50% of the treated bait compared to untreated bait. Squirrels were observed to be particularly reluctant to feed on Ramik Green^(R).

Long-term control of ground squirrels was not achieved in the field through the use of rodenticides. Ramik Green^(R) achieved 71% reduction in the rodent population in 3 weeks, but when diphacinone in oat groats was tested at the same concentration (0.005% A.I.) nearly 100% reduction occurred within two weeks. The reduction in squirrel population was accompanied by an increase in fleas on the surviving animals. Also, a substantial increase in the squirrel population was observed following removal of the rodenticide bait. Within six weeks squirrel density exceeded the level prior to treatment.

CONCLUSIONS:

Surveillance efforts have been successful in detecting serological evidence of plague infection among animals at Fort Hunter Liggett. The incidence of rodent and flea species surveyed at the installation conforms with the pattern established earlier. The high densities of fleas and rodents present a high potential for plague transmission, particularly in view of the serological evidence indicating that the disease is in the area. Although oral systemic insecticides appeared

useful in reducing fleas on squirrels, continuous application is required to maintain effective flea control. Removal of the poison bait has resulted in a rapid increase in the flea population. The effective use of anticoagulant rodenticides appears to require continuous application to insure that squirrels do not become re-established in the treated area. Also, their use must be preceded by flea control since the observed rise in flea density, in the experimental plots, points to the potential hazard to personnel entering the treated areas.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OC 6783	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	61102A	3M161102BS02				067	
XXXXXXXXXX	62759A	3A762759A831				004	
XXXXXXXXXX	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 - Biochemistry; 010100 - Microbiology; 003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PREVIOUS		B. FUNDS (In thousands)	
B. NUMBER:				76		5	
C. TYPE: Not Applicable				FISCAL YEAR		77	
D. KIND OF AWARD:				CURRENT		1.3	
E. CUM. AMT.				77		3	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Dermatology Research			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, John E., COL, MC				NAME: Lancaster, Michael V., CPT, MS			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5455			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Foye, J., SFC			
				NAME: Jaeger, J., DAC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Skin; (U) Pathogenicity; (U) Enzymes; (U) Vaccine; (U) Fungal inhibitors							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. To elucidate biochemical mechanisms of pathogenesis in debilitating fungal skin infections in soldiers. 2. To determine the role of environmental factors in infection by dermatophytes. 3. To develop novel therapeutic and immunological measures in preventing infections.							
24. Evaluate effects of environmental factors on germination and growth of dermatophytes <u>in vitro</u> . 2. Evaluate effect on infection of CO ₂ due to occlusion of the skin. 3. Develop methods to purify sub-cellular fractions for use as immunizing agents.							
25. (U) 75 07 - 76 09. 1. Results indicate specific levels of CO ₂ and humidity may be required for infection. 2. Levels may be reached by occlusion over the infection site. 3. Investigations are being continued to determine which environmental factor or combination of factors may be required for infection. 4. Purification techniques are being applied to sub-cellular fractions of <u>T. mentagrophytes</u> to produce a purified "trichophytin" antigen.							

ABSTRACT

PROJECT NO.	3A762759A831	Other Tropical Medicine
WORK UNIT NO.	004	Biochemical Mechanisms of Pathogenesis of Fungal Skin Infections
STUDY NO.	1	Role of Exocellular Enzymes in Pathogenesis

The mechanism(s) of pathogenesis of dermatophyte infections are being studied. Environmental factors which may affect the pathogenicity of dermatophytes are being studied in vitro and in vivo. The effect of CO₂ concentrations and relative humidity on dermatophyte germination has been determined in vitro. Studies have shown a relationship between increased CO₂ concentrations on the skin and fungal infections. Isolation of antigenic material continues to be a major research project. Methods have been developed to determine purity of antigen preparations. Investigations to increase yield and simplify the extraction procedures are continuing.

BODY OF REPORT

WORK UNIT NO. 004

Biochemical Mechanisms of Pathogenesis of Fungal Skin Infections

STUDY NO. 1

Role of Exocellular Enzymes in Pathogenesis

PROBLEM:

Dermatophyte infections are prominent producers of medically debilitating lesions in soldiers. The predominant etiologic agent for such infections in U.S. Army personnel in the Republic of Vietnam has been identified as Trichophyton mentagrophytes var. granulares. Although there is a voluminous amount of literature written about these fungal infections, a paucity of information remains regarding the specific mechanisms of pathogenesis.

Environmental factors play an important role in initiating dermatophyte infections. Research has centered around (1) determining the effects of CO₂ and humidity on germination and growth in vitro; (2) determining the relationship between CO₂ concentrations resulting from occlusion and dermatophyte infection; (3) and observing the isolation of sub-cellular immunogenic fractions which could act as a vaccine, skin test antigen, or both.

RESULTS AND DISCUSSION OF THE RESULTS:

The effects of CO₂ and humidity on germination and growth of dermatophyte in vitro have been studied in the microenvironmental chamber. When grown under 4 to 10% CO₂, the organisms shift into a hypersegmented growth phase. The phase is similar to that observed in active infection. A relative humidity of 75% at 32°C is required for successful germination of T. mentagrophytes spores. The germination occurs between 18 to 30 hours. By using either the micro-environmental chamber or a larger environmental chamber, the effect of combined CO₂ concentration and relative humidity will be studied. The effect of increased CO₂ concentration on the skin on infection has been studied. Under occlusion the concentration of CO₂ increases two-fold. Partially occlusive tapes have been used to control the amount of CO₂ held next to the skin. Infection has been initiated more readily under total occlusion than under partial occlusion. The influence that each environmental change under occlusion has on infection remains unclear. An artificial occlusion will be attempted in vitro by altering each environmental parameter in turn. A glycoprotein fraction has proven to be an excellent skin test antigen in determining previous experience with the dermatophyte infections. Methods were developed to separate and visualize the components of

the fraction. Such techniques were necessary to establish a degree of purity for the antigens.

Nonreactive components have been removed from the partially purified antigen fraction and work is continuing to produce a homogeneous antigenic product.

CONCLUSIONS:

Progress is being made in understanding the role of environmental factors in the pathogenic mechanism(s) of dermatophytes. Isolating a pure component responsible for the immunogenicity of the dermatophytes is progressing.

RECOMMENDATIONS:

Studies on effects of occlusion and effects of environmental factors on infection in man and animals should continue. A 10 gram supply of a purified fungal antigen is necessary. After this is obtained, more specific antigens will be sought.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTN ^a	8B. SPECIFIC DATA CONTRACTOR ACCESS ^a	9. LEVEL OF SUM ^a
76 04 02	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62759A	3A762759A831		00	005		
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Genetic Aspects of Mosquito Repellency							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
005900 Environmental biology; 002600 Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 11		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER: ^a Not Applicable				76		1	
c. TYPE:				7T		36	
d. KIND OF AWARD:				FISCAL YEAR CURRENT		7	
e. AMOUNT:				77		0	
f. CUM. AMT.				0		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-2370			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Moussa, M.A., LTC, MS			
				NAME: POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Repellent; (U) Deet; (U) Diethyl toluamide; (U) Mosquito; (U) Anopheles; (U) Aedes; (U) Culex							
23. TECHNICAL OBJECTIVE. ^a 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To define the genetic factors which govern sensitivity of mosquitoes to repellents and to develop a standardized test strain of <u>Aedes aegypti</u> for use in screening and testing of repellents and improved formulations for use by military personnel.</p> <p>24. (U) Species and strains of mosquitoes will be tested and compared with respect to sensitivity to the repellent diethyl toluamide (deet). Inbred lines will be established from unlike strains of <u>Aedes aegypti</u>. Crossing and back-crossing experiments, cross-repellency tests, and morphological studies will be accomplished on deet-sensitive and deet-insensitive inbred lines.</p> <p>25. (U) 76 04 - 76 09 Median effective dosages of deet were determined for 18 strains of <u>Anopheles albimanus</u>, <u>Anopheles quadrimaculatus</u>, <u>Anopheles stephensi</u>, <u>Aedes aegypti</u>, <u>Aedes taeniorhynchus</u>, <u>Culex pipiens</u> and <u>Culex tarsalis</u>. Median sensitivity to deet was a stable characteristic of each strain, but individual mosquitoes within strains exhibited differing levels of sensitivity. Six inbred strains of <u>Aedes aegypti</u> were produced for use in further studies and for service as standardized repellent test strains. Seventy-two chemical compounds synthesized by Stanford Research Institute were evaluated for their potential repellency to mosquitoes. Studies will continue under a new work unit 015, Project 3M762772A810, Military Skin Disease.</p>							

^aAvailable to contractors upon originator's approval.

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762759A831

Other Tropical Medicine

WORK UNIT NO. 005

Genetic Aspects of Mosquito
Repellency

The dose response relationships of several strains and species of mosquitoes to the military standard repellent N,N-diethyl-m-toluamide (deet) have been established. Median effective dosages of deet were determined for Anopheles albimanus, Anopheles quadrimaculatus, Anopheles stephensi, 10 strains of Aedes aegypti, 2 strains of Aedes taeniorhynchus, 2 strains of Culex pipiens and Culex tarsalis. The variation observed was approximately 2-fold among strains of the same species and approximately 8-fold among strains of different species. Median sensitivity to deet was a stable characteristic of each strain, but individual mosquitoes within strains exhibited differing levels of sensitivity.

Six inbred strains of Aedes aegypti were produced by 10 generations of single-pair, brother-sister mating, and the strains produced are considered to be essentially isogenic. These strains will be used in further genetic studies and for service as standardized repellent test strains in the military repellent development program.

BODY OF REPORT

WORK UNIT NO. 005

Genetic Aspects of Mosquito
Repellency

PROBLEM:

The USAMRDC supports an extensive program of research on chemical repellents for the protection of military personnel from mosquito-borne diseases. Investigators evaluating candidate repellents frequently report disparate or conflicting results. It is believed that genetic factors relating to the test mosquitoes may account, in part, for those discrepancies. The present study provides for a quantitative assessment of the relative importance of environmental and genetic factors in determining the responses of mosquitoes to repellents and for the development of genetically homogeneous strains of the yellow fever mosquito, Aedes aegypti (Linnaeus) to use as the standardized repellent test strains.

RESULTS AND DISCUSSION OF THE RESULTS:

The dose-response relationships of several strains and species of mosquitoes to the military standard repellent N,N-diethyl-m-toluamide (deet) have been established. The median effective dosages (ED₅₀) of deet were determined for Anopheles albimanus, Anopheles stephensi, Anopheles quadrimaculatus, 10 strains of Aedes aegypti, 2 strains of Aedes taeniorhynchus, 2 strains of Culex pipiens and Culex tarsalis. The ED₅₀ values obtained for strains of the same species were 0.024 to 0.042 mg/cm² for 10 strains of Aedes aegypti, 0.015 and 0.018 mg/cm² for 2 strains of Aedes taeniorhynchus, and 0.011 and 0.017 mg/cm² for 2 strains of Culex pipiens. The differences observed among strains of different species were substantially greater than the intraspecies differences. The extremes were 0.011 mg/cm² for the University of California Davis strain of Culex pipiens and 0.076 mg/cm² for Anopheles albimanus. Such differences among species and strains tested under standard conditions constitute presumptive evidence of genetic involvement in the responses of mosquitoes to deet.

Four strains of Aedes aegypti (AMPHUR, MOYO INDOOR, MASAKA, and OCALA) were re-tested after the passage of several generations, and the linear correlation of the results of the later tests with those of the original tests was calculated. The analysis indicated that 90% of the variation observed in ED₅₀ values could be attributed to stable genetic differences among the strains. The remaining 10% of the variation was attributable to "nongenetic" or "environmental" effects, including the experimental and sampling error, the genetic drift and certain interactions of genetic and environmental factors.

The dose-response relationship determined by the ED_{50} test provided a measure of intra-strain heterogeneity with respect to the deet-sensitivity trait. For example, it is possible to calculate from our data that 22, 48, 75 and 92% of the MOYO INDOOR strain of Aedes aegypti were repelled by dosages of 0.02, 0.04, 0.08 and 0.16 mg/cm² of deet, respectively. Thus, when the mosquitoes were reared and tested under standard conditions, certain individuals within the strain were relatively sensitive to deet and others were relatively insensitive to it.

From the foregoing results and discussion it is clear that the responses of mosquitoes to deet are subject to genetic control and that a potential exists, both among and within strains, for the isolation of genetically homogeneous lines of mosquitoes offering substantially reduced variation in the deet-sensitivity trait. Such lines would be useful as foundation stock for standardized repellent test strains. If the lines were disparate with respect to the deet-sensitivity trait, other areas of research would have been opened; and then the mode of inheritance of the trait, linkages between sensitivity to deet and sensitivity to other repellents, and the site of repellent action in the mosquito could be determined.

Ninety inbred lines of the MASAKA strain ($ED_{50} = 0.024$ mg/cm²) and the MOYO INDOOR ($ED_{50} = 0.042$ mg/cm²) were initiated. Six lines were successfully carried through the complete inbreeding program. Losses of lines in the mosquito inbreeding program averaged 24% per generation. These losses were due, in part, to deleterious inbreeding effects and, in part, to technical difficulties in the management of small single-brood colonies. Several apparent effects of inbreeding included dwarfism, defective wing folding, reduced antennal hairs, sex ratio distortion and failure to feed, oviposit, hatch or develop. Nongenetic losses were reduced, to the extent possible, by retaining redundant representatives of the various lines and by improving our procedures for handling single pairs and single broods.

The 6 inbred lines of Aedes aegypti produced in the program are currently being brought to colony strength. These 6 strains (designated as strains R-3, R-13, R-21, R-44, S-15 and S-22) are nearly 100% isogenic. Final selection of strains for retention for use as standardized repellent test strains will be based in part on level of sensitivity to deet and in part on considerations of viability, ease of rearing, and ease of handling.

Chemical compounds synthesized by Stanford Research Institute (SRI) under contract with USAMRDC were evaluated for their repellency to mosquitoes. To date, 72 compounds have been tested for repellency to the University of California San Francisco strain of Aedes aegypti. The median effective dosages of these chemicals ranged from 0.8 ug/cm² upward, including one compound which had no detectible effect. Thus, our baseline data on this species effectively cover its entire known

range of genetically determined avoidance responses to chemicals. The correlation of these responses with chemical structure and properties is currently being studied by SRI.

CONCLUSIONS:

Mosquito avoidance of repellents is subject to a substantial degree of genetic control. Within-strain variation in the avoidance response can therefore be reduced by minimizing genetic heterogeneity in the strain. The 6 inbred strains of Aedes aegypti produced during the year provide a unique resource for detailed studies of the genetic aspects of mosquito repellency and for the selection of improved, standardized mosquito repellent test strains.

RECOMMENDATIONS:

Inbred strains of Aedes aegypti produced in this study should be brought to strength and tested to determine their respective levels of response to deet. Two of the more disparate strains should be retained for use in further genetic studies and as standardized repellent test strains.

PUBLICATIONS:

Rutledge, L.C., M.A. Moussa, and C.J. Belletti. An in vitro blood-feeding system for quantitative testing of mosquito repellents. Mosquito News, 36:283-293, 1976

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTN ^a	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62759A	3A762659A831		00		006	
b. XXXXXX							
c. XXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a (U) Experimental Fungus Infections in the Skin of Man: A Therapeutic Model (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 010100 - Microbiology; 003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
69 11		76 09		DA		C. In-House	
17. CONTRACT / GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		76	
b. NUMBER:				FISCAL		77	
c. TYPE: Not Applicable				CURRENT		77	
d. AMOUNT:				77		0	
e. KIND OF AWARD:				77		0	
f. CUM. AMT.				77		0	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
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RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Greenberg, J.H., LTC, MC			
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22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
23. KEYWORDS (Precede EACH with Security Classification Code) (U) Laboratory Animals; (U) Dermatophytosis; (U) Experimental Infection; (U) Skin; (U) Human Volunteers							
24. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) 1. To identify antigens from dermatophytes (both cellular and extra-cellular) that have immunizing potential for a vaccine for soldiers. 2. To evaluate prophylactic and therapeutic antifungal agents. 3. To develop in guinea pigs a state of non-recognition or tolerance of dermatophytes and their antigens. 4. To test antimetabolites and other chemicals and their effects on dermatophyte infections.							
24. (U) 1. Antigens extracted from dermatophytes will be tested in "experienced" guinea pigs and their lymphocytes. Those that are most antigenic (best reactions) will be evaluated for their ability to stimulate cell mediated immunity (CMI). 2. Therapy studies will be performed in "inexperienced," tolerant and immune suppressed guinea pigs. 3. Guinea pigs will be feed, injected interperitoneally or intravenously with large amounts of fungus antigen for inducing tolerance. The influence of antimetabolites and histamine on fungal infections, T and B cells, and lymphocyte transformation will be evaluated.							
25. (U) 75 07 - 76 09 1. The quantitative dermatophyte infection in guinea pigs has been utilized for further studies of immunity, immunological manipulation, histopathological studies, histamine influence studies, and prophylactic and therapeutic antifungal studies.							

ABSTRACT

PROJECT NO. 3A762659A831 Other Tropical Medicine
TASK NO. 00
WORK UNIT NO. 006 Experimental Fungus Infections
in the Skin of Man: A therapeutic Model

The following investigations have been conducted under this work unit:

- STUDY NO. 7 The Influence of Cyclophosphamine in Dermato-
phyte Infections, (A further Investigation)
- STUDY NO. 9 The Histopathology Examination of Skin Test and
Infection Sites (A further Investigation)
- STUDY NO. 10 Histamine Effect on Cell Immunity In vivo
- STUDY NO. 11 Inhibition of Keratinase and its Influence on
Infectivity of Trichophyton Mentagrophytes
- STUDY NO. 12 Therapeutic and Prophylactic Effects of Antifungal
Agents (Topical)

Studies No. 7, 9, 10, 11, and 12: Over the last year, we used our newly developed animal infection model for investigating multiple parameters of infection and immunity. We studied the immunity gained through infection, means of inhibiting these infections, and/or means of changing their course. We studied agents that influence the developed immunity, and investigated the histopathology of the infection and skin test site, and how they are influenced by immunologic manipulation. We evaluated the fungus-host relationship and attempted to evaluate the importance of keratinase in the infecting process. Using this model, we evaluated antifungal agents both prophylactically and therapeutically.

We found the following: all antigens (cellular and exocellular which have been separated by us and give a positive skin test in immune animals) show cutaneous basophilic hypersensitivity (CBH) on histopathologic examinations. Cyclophosphamide, given prior to infection, changes the CBH skin test to classic tuberculin-like delayed hypersensitivity (CDH). This change in skin test morphology does not reflect any change in the infection's course which is identical in cyclophosphamide and control animals. Histamine blocks CBH and CDH skin tests and this may explain why individuals with immediate hypersensitivity to trichophytin often have chronic infections. Keratinase may not be necessary for skin invasion in in vivo but we have no in vivo way of

determining whether or not we have truly inhibited the production of keratinase. We have had minimal success by using this model to predict antifungal prophylactic and therapeutic efficacy.

BODY OF REPORT

WORK UNIT NO. 006

Experimental Fungus Infections in
the Skin of Man: A Therapeutic
Model

STUDY NO. 7

The Influence of Cyclophosphamide
in Dermatophyte Infections (A fur-
ther Investigation)

PROBLEM:

We pointed out in our last annual report (FY 75, pp. 223-224) that cyclophosphamide, in small doses, could lead to chronicity in experimentally induced dermatophyte infections. We also noted that a large initial dose did not influence this infection. We interpreted this as further proof of the insignificant role of B lymphocytes in dermatophyte immunity. We have now looked at the histopathology of trichophytin skin tests in animals that received larger initial doses of cyclophosphamide compared to an infected control group.

The histopathologic study of skin tests is important in the screening of antigens. These may have future potential as vaccines to be used for protection in military personnel assigned to high risk areas.

RESULTS AND DISCUSSION OF RESULTS:

The animals receiving large doses of intraperitoneal cyclophosphamide showed the same course as normal control animals. However, skin testing of the cyclophosphamide (Cy) animals elicited a cutaneous basophilic hypersensitivity (CBH) rather than the expected classic delayed hypersensitivity (CDH) response. This is in agreement with our interpretation that B lymphocytes are not necessary for dermatophyte immunity; that CBH must be a B lymphocyte influenced form of CDH; and that, as an index of immunity, CBH seems to be as adequate as CDH.

CONCLUSIONS:

Cy can convert CBH to CDH but this does not influence the outcome or course of the infection. As an index of immunity CBH seems to be as adequate as CDH.

RECOMMENDATIONS:

It is still necessary to evaluate the reason for chronic infection in small dose Cy group and to evaluate this as a model for trial of therapeutic agents.

PUBLICATIONS:

This work was presented to the Society of Investigative Dermatologists in Atlantic City, N.J., April 30, 1976. This work was summarized in a news article in The Skin and Allergy News of July 20, 1976.

Greenberg, J.H., S. Kerbs, R. Fields, R.D. King: Trichophytin Reactions-Further Investigations on Cutaneous Basophilic Hypersensitivity. Presented SID April 30, 1976.

STUDY NO. 9

The Histopathologic Examination
of Skin Test and Infection
Sites (A further Investigation)

PROBLEM:

Last year, we reported the histopathologic reaction seen in infection and trichophytin skin test sites. We found that trichophytin injected sites developed cutaneous basophilic hypersensitivity (CBH). We wondered if the histopathologic discrepancy between the trichophytin site (CBH) and the infection site, i.e. classic delayed hypersensitivity (CDH) might indicate that the antigen we were using did not adequately represent the antigen experienced by the guinea pig during an infection. Another possible explanation for this difference might have been the difference in antigen presentation. The infection was percutaneous and the skin test intradermal. To explain this apparent discrepancy, we have tried a large number of antigens, including purified crude and non-purified, cellular and exocellular antigens. We have also done a series of skin tests applying the antigen percutaneously.

This study grew out of our continuing effort to identify antigens which have immunizing potential. These could be used to vaccinate military personnel assigned to high risk areas.

RESULTS AND DISCUSSION OF RESULTS:

Over the last year, we have isolated a number of antigens from Trichophyton mentagrophytes var asteroids and T. mentagrophytes var granulosum. These antigens have varied from crude to highly purified and from cellular to exocellular. In every case, when the immune animal was tested with the antigen, a CBH response was noted. This reaction was noted in those animals exposed to antigen either percutaneously or intradermally. The modes of antigen presentation do not seem to influence the cellular infiltrate. We also performed histamine assays on both CBH and CDH biopsy sites and on the infection sites. We found no significant difference between CBH and CDH sites but infection sites were much lower in histamine content.

CONCLUSIONS:

All antigens tested, whether extremely crude or purified, caused CBH reactions. Presentation of the antigen did not seem to be an important factor in determining this cellular reaction. At this time, we do not feel as strongly as we did previously that the difference in morphology between skin test and infection site indicates that we have the wrong antigen.

RECOMMENDATIONS:

Further work should be undertaken to explain what causes CBH and if it actually predicts antigens that would not make good vaccines.

PUBLICATIONS:

This work was presented to the American Federal Clinical Research, in Carmel, CA 5-7 February, 1976.

Greenberg, J.A., S. Kerbs, R.D. King, and R. Field: Trichophytin Reactions-Classical Delayed Hypersensitivity or Cutaneous Basophilic Hypersensitivity. Clinical Res 24:96A, 1976.

STUDY NO. 10

Histamine Effect on Cell
Immunity In vivo

PROBLEM:

The observation has been made that individuals with chronic dermatophyte infections have immediate trichophytin skin test reactions and also commonly have an atopic diathesis. It has been observed that histamine in vitro inhibits cell mediated immunity (CMI). This study was undertaken to determine the in vivo effects of histamine on skin test reactivity. The understanding of the mechanisms of chronic dermatophyte infections and the possibility that this will lead to a rational therapy may be important in keeping certain individuals from assignment to endemic areas and also may help the predicted 10-15% military personnel with chronic infections.

RESULTS AND DISCUSSION OF RESULTS:

Guinea pigs had jugular catheters inserted and then were infused with histamine. Intradermal injections of trichophytin and purified protein derivative (Ppd) were given 16 hours after the start of the infusion. These skin tests were negative at 24 and 48 hours. This indicates that increased histamine levels in vivo can block CMI.

CONCLUSIONS:

Histamine given intravenously can block skin test reactivity and possibly CMI.

RECOMMENDATIONS:

It is necessary to keep the infusion going for 2 weeks and determine if the histamine will cause a change in the infection course.

PUBLICATIONS:

None.

STUDY NO. 11

Inhibition of Keratinase and its
Influence on Infectivity of Trichophyton Mentagrophytes

PROBLEM:

Trichophyton mentagrophytes var granulosa has the ability to elaborate many exoenzymes. An enzyme felt to be important in dermatophyte infections is keratinase, an enzyme that digest keratin and makes it available as a nutriment for the dermatophyte. In the presence of glucose, this enzyme is not produced. If this enzyme is the pathogenic factor of T. mentagrophytes, then its inhibition should prevent the development of an infection. This finding would lead to an entirely new treatment and vaccination program. It would allow us to treat military personnel in endemic dermatophyte areas with enzyme inhibitors both prophylactically and therapeutically.

RESULTS AND DISCUSSION OF RESULTS:

Guinea pigs were infected in the usual manner. A glucose solution was applied to the infection site twice daily from the time of application. A non-glucose solution was applied to the control group. Animals in each group manifested no difference each having standard infections. This tends to eliminate the necessity of keratinase for pathogenesis. We cannot say this with certainty since we have no methods of measuring keratinase in vivo and therefore cannot rule out its production, even in the presence of glucose.

CONCLUSIONS:

Keratinase may not be necessary as a factor for pathogenesis.

RECOMMENDATIONS:

Mutants of T. mentagrophytes that do not produce keratinase should be developed and the pathogenicity of this organism should be investigated.

PUBLICATIONS:

None.

STUDY NO. 12

Therapeutic and Prophylactic Effects of Antifungal Agents (topical)

PROBLEM:

Most investigators, other than those attached to a pharmaceutical company, use one of two methods for antifungal testing, either (1) with human volunteers, or (2) with stratum corneum samples. The use of guinea pigs for these studies may be easier and less time-consuming.

Prophylactic and therapeutic agents are necessary to use on military personnel in endemic areas and those in nonendemic areas that develop a random infection.

RESULTS AND DISCUSSION OF RESULTS:

Multiple antifungal agents were used for prophylaxis and for therapy. These agents included miconazole, chlortrimazole, thiobenzadole, griseofulvin, tolnaftate, haloprogin, and vehicle. In our investigation, each was applied according to the following schedule. For prophylaxis, the animals were treated for 7 days with one of the agents (twice per day application). They then were inoculated with 100, 10,000, or 1,000,000 spores. Therapeutically, the drugs were applied twice a day either from the time of first noting the infection or at its maximum (Day 10-11). At 100 spore inoculans, drugs except haloprogin were good prophylactically; at 10,000 spores, tolinaftate, miconazole, and griseofulvin were effective; and at 1,000,000 spores, only tolfanate seemed effective. Therapeutically, it was hard to judge efficacy because of the limited duration of the infection and the criteria of improvement were difficult to measure.

CONCLUSIONS:

The experimental infection seems to lend itself to testing of prophylactic agents. With different inocula, the degree of efficacy can be measured. The model now is not adequate for therapeutic studies and more prolonged infections must be obtained.

RECOMMENDATIONS:

More work on chronic infections needs to be done.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISB'N INSTR'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 06 30	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62759A	3A762759A831	00	007			
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code)*							
(U) Colonization of Selected Insect Vectors of Disease							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
005900 Environmental Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 11		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		76	
b. NUMBER* Not Applicable				FISCAL		1.5	
c. TYPE:				CURRENT		4	
d. AMOUNT:				77		0	
e. KIND OF AWARD:				0		0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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				SOCIAL SECURITY ACCOUNT NUMBER:			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Applicable				NAME: Moussa, M.A., LTC, MS			
				NAME: POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Mosquito; (U) Sand fly; (U) Flea; (U) Tick;							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To establish and maintain colonies of selected insects of military medical importance and to develop improved methods for the laboratory production of insect resources for support of repellent and disease transmission studies.</p> <p>24. (U) Laboratory-adapted or field-collected strains of selected insects are obtained and reared. Improved methodology is developed, based on studies of the reproductive potential and the biological requirements and tolerances of the species colonized.</p> <p>25. (U) 75 07 - 76 09 Colonies of 7 species of mosquitoes, a phlebotomine sand fly, 2 species of fleas and a tick were established. The flea colonies are original and the sand fly colony is the first of its kind in the U.S. New methods were developed for the rapid counting of mosquito larvae and for the <u>in vitro</u> blood-feeding of mosquito adults. Novel techniques for sand fly culture were tested and mass production trials were initiated. These studies will continue under a new work unit 015, Project 3M762772A810, Military Skin Disease. Publications - see Mosquito News 36: 200, 1976.</p>							

*Available to contractors upon originator's approval.

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762759A831 Other Tropical Medicine
WORK UNIT NO. 007 Colonization of Selected
Insect Vectors of Disease

Colonies of 7 species of mosquitoes (Anopheles quadrimaculatus, Anopheles albimanus, Anopheles stephensi, Aedes aegypti, Aedes taeniorhynchus, Culex pipiens and Culex tarsalis), a phlebotomine sand fly (Lutzomyia longipalpis), 2 species of fleas (Diamanus montanus and Hoplopsyllus anomalus) and a tick (Dermacentor variabilis) were established and maintained in the insectary for use in repellent research, disease transmission, and related studies. The 2 flea species have not been previously colonized. The sand fly colony is the first of its kind in the U.S.

Significant improvements were made in laboratory methods for the production of mosquitoes and sand flies, e.g., rapid counting methods for mosquito larvae were developed, an improved in vitro mosquito blood-feeding system was designed and tested, new techniques for sand fly culture were tested, and mass production trials for sand flies were initiated. Research on insect colonization methods are being continued to provide a comprehensive in-house resource for use in the military repellent development program.

BODY OF REPORT

WORK UNIT NO. 007

Colonization of Selected
Insect Vectors of Disease

PROBLEM:

Laboratory investigations relating to the development of improved insect repellents depend on the availability of an adequate stock of representative insect species for experimental use. Laboratory colonies provide an economical in-house resource for repellent bio-assay in lieu of travel to distant areas where important species are known to occur. The present study encompasses research relating to the development of methods for the laboratory production of experimental insects required for the repellent development program. Emphasis is placed on the development of practical, efficient methods for the mass-production of selected, representative species.

RESULTS AND DISCUSSION OF THE RESULTS:

Four new species of mosquitoes were colonized in the insectary during the year. The species now available for use are (1) Anopheles (Anopheles) quadrimaculatus Say (2 strains), the historical vector of malaria in Eastern North America, (2) Anopheles (Nyssorhynchus) albimanus Wiedemann, the major vector of malaria in Central and South America, (3) Anopheles (Cellia) stephensi Liston, a major vector of malaria in Southern Asia and the Middle East, (4) Aedes (Stegomyia) aegypti (Linnaeus) (4 strains), the primary vector of yellow and dengue fevers, (5) Aedes (Ochlerotatus) taeniorhynchus (Wiedemann) (3 strains), a major pest species on the East and West Coasts of the U.S., (6) Culex (Culex) pipiens Linnaeus, the primary vector of periodic Bancroftian filariasis and a major vector of viral encephalitis, and (7) Culex (Culex) tarsalis Coquillett, a major vector of viral encephalitis and a major pest species in the Western U.S. These species were selected for colonization on the basis of importance to Military Preventive Medicine, taxonomic distance, and ease of rearing and handling. Five of the 7 species appear on a list of the 10 most important mosquito species of the U.S. prepared by the American Mosquito Control Association, and the 2 others (Anopheles albimanus and Anopheles stephensi) are major vectors of malaria in overseas areas. To our knowledge, this battery of test species is the most extensive and comprehensive to be assembled for use in repellent research.

A careful balance of larval numbers with water surface, water volume and diet is required for survival and uniform, optimal growth and development of mosquito larvae. Two new methods of estimating numbers of mosquito larvae were developed and tested during the year. One method makes use of a specially prepared set of photographic standards, while the other is based on a tendency of mosquito larvae

to swim downward when confined in a narrow tube. Both methods are simpler and more rapid than any of the methods previously available.

For economic, humane, and practical reasons, several laboratories in the U.S. and elsewhere have developed in vitro blood-feeding systems for use in lieu of laboratory animals for mosquito colony maintenance. The development of an in vitro blood-feeding system suitable for use in the LAIR insectary was initiated. The apparatus currently under trial is a plastic, modular, water-warmed configuration designed to service varying numbers and sizes of mosquito cages simultaneously. Trials of various types of membranes for use with the blood-feeding apparatus have established that Parafilm "M"™ @ \$0.14/ft² is an acceptable substitute for other materials (Baudruche membrane @ \$2.62/ft², lambskin @ \$3.38/ft²) that have been used. It is anticipated that the present system will be placed in service in the near future; however, further refinements are desirable, and research in the area will be continued.

A colony (initiated in May 1975) of the phlebotomine sand fly Lutzomyia longipalpis (Lutz and Neiva), a major vector of leishmaniasis in South America, was conclusively established in the insectary during the year. It represents the first colony of a New World sand fly species to be established in the U.S. Because of their relatively narrow tolerances, low fecundity and long life cycle, the phlebotomine sand flies are among the most difficult insects to breed in the laboratory; mass rearing techniques have not yet been developed for any species. Four major advances were made during the year. (1) Commercial organic garden compost was identified as a superior larval rearing medium. (2) Disparate tolerances were recognized for the immature stages (warm, moist preference) and the adult stage (cool, dry preference). (3) Routine survival of the adult females to 2 or more gonotrophic cycles was achieved. (Nearly 100% post-oviposition mortality has been reported by other workers.) (4) The initial mass production trials were completed. These trials demonstrated that all the life stages of the species are amenable to intensive culture in large systems. Further modifications and refinements of the initial culture methods are being pursued to provide large numbers of sand flies for mass production on a continuous basis.

Colonies of the flea species Diamanus montanus Baker and Hoplopsyllus anomalus Baker were established from material collected in the field at Fort Hunter Liggett, California. These fleas are ectoparasites of the California ground squirrel Spermophilus beecheyi (Richardson) and both have been implicated in the transmission of sylvatic plague. To our knowledge, these are the first colonies of these particular species to be established. Initial difficulties with Hoplopsyllus anomalus were resolved by providing drier conditions of culture, in accord with conditions observed in the field at the time of its peak of abundance.

A colony of the ixodid tick Dermacentor variabilis (Say), a major vector of Rocky Mountain Spotted Fever and tularemia, was established from material obtained from the U.S. Department of Agriculture Animal Insects Laboratory, Kerrville, Texas, in December 1975. The long life cycle (ca. 200 days) is offset by an exceptional fecundity (ca. 2000 eggs per female), and no significant difficulty has been experienced in maintaining the colony.

CONCLUSIONS:

A comprehensive battery of test species for use in repellent research has been established. Two species of fleas have been colonized for the first time from material collected in the field. Significant advances have been made in laboratory production methods for mosquitoes and sand flies.

RECOMMENDATIONS:

Research on insect rearing methods should be continued with a view toward effecting further economies and benefits for the repellent development program. Emphasis should be placed on improved methods for rearing mosquitoes, because of their usage and importance, and on improved methods for rearing sand flies because of their difficulty and relatively high cost in manpower.

PUBLICATIONS:

Rutledge, L.C., G.N. Piper and M.A. Moussa. Initiation of a Neotropical Sand Fly Colony in U.S. Mosquito News, 36:200-202, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY 76 04 02	4. KIND OF SUMMARY H. Termination	5. SUMMARY SCTY ^a U	6. WORK SECURITY ^a U	7. REGRADING ^a NA	8A. DISB'N INSTR'N NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62759A	3A762759A831		00	008		
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code) ^a (U) Ultrastructural Studies of the Host-Parasite Relationships in Cutaneous Leishmaniasis							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 010100 Microbiology; 002600 Biology							
13. START DATE 75 03		14. ESTIMATED COMPLETION DATE 76 09		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		.5	
b. NUMBER: Not Applicable				FISCAL YEAR		.1	
c. TYPE:				CURRENT		22	
d. AMOUNT:				77		0	
e. KIND OF AWARD:				0		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Childs, G.E., CPT, MS			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-3564			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Moussa, M.A., LTC			
				NAME: Rutledge, L.C., DAC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Cellular Immunology; (U) Leishmania enriettii; (U) Delayed Hypersensitivity; (U) Electron Microscopy; (U) Immediate Hypersensitivity							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To study the host parasite relationships in cutaneous leishmaniasis with emphasis on the interrelationships of the various cells of the cell-mediated response. Information derived from this study will provide a better understanding of the pathogenesis and immune response which contribute to the resolution or complication of the infection.</p> <p>24. (U) Infections of <u>Leishmania enriettii</u> in guinea pigs will be utilized as a model system in this study. Inoculation sites of lesions and lymph nodes will be fixed for electron microscopy. Changes in the cells of the immune system and their interactions related to the infection will be observed. Delayed hypersensitivity reactions produced in immune guinea pigs by <u>Leishmania</u> or leishmanial antigens will also be examined.</p> <p>25. (U) 76 04 - 76 09 Cytochemical and ultrastructural observations revealed acid mucopolysaccharides on the periphery of the parasites which suggests mucopolysaccharides may be responsible for immediate skin reactions in human infections. The <u>L. enriettii</u>-guinea pig model has proven useful in delineating the role of amastigotes and promastigotes in inducing immediate and delayed hypersensitivity responses. Amastigote antigens elicited a strong immediate response with no subsequent delayed reaction in contrast to a slight immediate response and strong delayed response for promastigote antigens. When antihistamines were used in conjunction with amastigote antigen, the immediate response was blocked and a strong delayed response appeared.</p>							

^aAvailable to contractors upon originator's approval.

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DD FORM 1498

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ABSTRACT

PROJECT NO. 3A762759A831

Other Tropical Medicine

WORK UNIT NO. 008

Ultrastructural Studies of the
Host-Parasite Relationships in
Cutaneous Leishmaniasis

Ultrastructural and cytochemical examination have confirmed the presence of a mucopolysaccharide surface coat on the periphery of amastigotes of Leishmania enriettii. This surface coat may be analogous to the exo-antigens believed associated with the immediate skin reaction in human infection. The L. enriettii-guinea pig model has proven useful in delineating the role of amastigote and promastigote antigens in inducing immediate and delayed hypersensitivity responses. Amastigotes elicited a strong immediate response with no subsequent delayed reaction in contrast to promastigotes. When antihistamine was used in conjunction with amastigotes, the immediate response was blocked and a strong delayed reaction appeared within 48 hours. Results obtained in these investigations are essential to the development of a skin test for the detection of active or post leishmanial infection among military personnel serving in, or returning from, endemic areas.

BODY OF REPORT

WORK UNIT NO. 008

Ultrastructural Studies of
the Host-Parasite
Relationships in Cutaneous
Leishmaniasis

PROBLEM:

Cutaneous leishmaniasis is a disfiguring infection encountered by military personnel stationed in endemic areas. The disease is caused by intracellular protozoan parasites of the genus Leishmania. Immunity in most forms of leishmaniasis is conferred principally by cell-mediated responses. The exact nature of these responses and the mechanisms by which lymphocytes and other cell types interact to recognize and destroy the parasite are not fully understood. Ultrastructural studies of host-parasite relationships in a laboratory model will provide a better understanding of the pathogenesis and immune responses which contribute to the prognosis of infection.

RESULTS AND DISCUSSION OF THE RESULTS:

A study to relate subcellular changes in cells of the immune response of guinea pigs to resolution of cutaneous infection was continued. Several attempts to locate parasites and/or lymphocytes by electron microscopy in lesions on Days 1 and 4 were not successful.

Ultrastructural examination and cytochemistry of L. enriettii amastigotes and promastigotes with stains specific for acid mucopolysaccharides revealed a thick layer on the periphery of the amastigote and a thin layer on the promastigotes with cytoplasmic extensions. This material appears to exist as a surface coat and may be analogous to exo-antigens believed to be associated with the immediate skin reaction in human infections.

Delayed hypersensitivity responses in guinea pigs to leishmanial antigens were also examined. A strong immediate response was observed with L. enriettii amastigote antigens. This was in contrast to promastigote antigen which gave a slight immediate reaction followed by a pronounced delayed response. When antihistamine was used in conjunction with amastigote antigens, the immediate response was blocked and strong delayed response appeared within 48 hours.

CONCLUSIONS:

The presence of a mucopolysaccharide surface coat on the periphery of amastigotes has been confirmed by ultrastructural and cytochemical examination. It is suspected that this surface coat is analogous to exo-antigens believed associated with the immediate skin reaction. The L. enriettii-guinea pig model has proven useful in delineating the role of amastigote and promastigote antigens in inducing immediate and delayed responses.

RECOMMENDATIONS:

Emphasis should be re-directed toward investigations of leishmanial antigens for inducing immediate and delayed skin responses relative to diagnosis and prognosis of leishmaniasis. Ultrastructural studies should focus on providing additional evidence on the role of the surface coat in these hypersensitivity reactions.

Studies formerly conducted under this work unit will continue under a new work unit 015, entitled "Diagnosis and Prevention of American Leishmaniasis in Military Personnel," Project 3M762772A810, Military Skin Disease.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6095	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISB'N INST'N	9a. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A810				010	
b. XXXXXXXX	62759A	3A762759A831				010	
c. XXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Microbial Interactions on Healthy and Infected Skin of Soldiers							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
010100 - Microbiology, 003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 04		CONT		DA		In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER:				76		2.2	
c. TYPE: Not Applicable				77		.5	
d. AMOUNT:				CURRENT		15	
e. KIND OF AWARD:				77		2.2	
f. CUM. AMT.						66	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Cutaneous Infection Division			
				Department of Dermatology Research			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Keith, William, Jr., CPT, MS			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Normal Flora; (U) Skin Ecology; (U) Microbiology;							
(U) Human Skin; (U) Human Volunteers							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Lessons learned during the Viet Nam conflict showed that 262,126 man-days were lost due to skin problems. This work unit addresses the problem of why some soldiers are resistant while most are susceptible to epidemic pyoderma and fungal infections with the objective of developing prophylactic measures against these infections and thus reduce troop non-effectiveness.</p> <p>24. (U) The objective can be attained by surveys for identification and quantitation of skin microorganisms on healthy and infected human subjects, by developing an <u>in-vitro</u> model for microbial ecological interactions, and by experimentally altering skin flora in prophylactic and therapeutic studies.</p> <p>25. (U) 76 04 - 76 09 An antibiotic-producing strain of <u>Bacillus licheniformis</u> survived longer and at higher levels on human skin than control strains. Individuals could be grouped into long and short-term carriers of Bacilli. On eczematous lesions <u>Staphylococcus aureus</u> >10⁵ colony-forming units/cm² seemed to reduce surrounding flora, and when >10⁶ cfu/cm², it constituted almost 100% of total aerobic flora. Interactions of <u>B. licheniformis</u> and representative skin bacteria were examined in the Ecolo Gen; results corroborated <u>in vivo</u> data. A simple agar medium was developed to differentiate <u>Serratia</u> from other <u>Enterobacteriaceae</u>. A commercial antimicrobial deodorant soap did not decrease the population of bacteria on the forearm but altered the composition of normal flora. Skin flora maps, developed as a tool for studying skin ecology, demonstrated that when wet-occlusive dressings are removed, the underlying flora affects the composition of flora in distant areas. The development of arthrospores of <u>Trichophyton mentagrophytes</u> were examined by transmission and scanning electron microscopy.</p>							

^aAvailable to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762759A831 Other Tropical Medicine
WORK UNIT NO. 010 Microbial Interactions on
Healthy and Infected Skin of
Soldiers

The following investigations have been conducted under this Work Unit.

STUDY NO. 2 Interactions of Cutaneous Microorganisms In Vivo

STUDY NO. 3 Interactions of Cutaneous Microorganisms In Vitro

STUDY NO. 4 Alteration of Skin Flora by Soaps

STUDY NO. 5 Skin Flora Maps

STUDY NO. 6 Morphological Studies of Dermatophytes and
Dermatophytosis

Study No. 2. The survival of Bacillus licheniformis on human skin was studied. Individuals could be differentiated into long- and short-term carriers. Bacitracin-producing ATCC 10716 survived significantly longer and at higher levels on short-term carriers than control strains. In eczematous lesions Staphylococcus aureus, at densities $>10^6$ cfu/cm², constituted almost 100% of total aerobic flora.

Study No. 3. Interactions of B. licheniformis and representative skin bacteria were examined in the EcoloGen. Results corroborated in vivo data. A simple agar medium based on the hydrolysis of Tween 80 was developed to differentiate Serratia, including non-pigmented strains, from other Enterobacteriaceae.

Study No. 4. A commercial antimicrobial deodorant soap did not decrease the population of bacteria on the forearm, but it reduced diptheroids in 71% of carriers, simplified the composition of flora, and favored Acinetobacter calcoaceticus and Micrococcus luteus.

Study No. 5. Skin flora maps were developed as a tool for the study of skin ecology. When wet-occlusive dressings were removed, the underlying flora profoundly affected the composition of flora in distant areas.

Study No. 6. The development of arthrospores of Trichophyton mentagrophytes was examined by transmission and scanning electron microscopy.

BODY OF REPORT

WORK UNIT NO. 010

Microbial Interactions on Healthy
and Infected Skin of Soldiers

STUDY NO. 2

Interactions of Cutaneous Microorganisms
In Vivo

PROBLEM:

The in vitro production of antibiotics by skin microorganisms has been reported, but whether such synthesis occurs on the stratum corneum or is important to skin ecology has not been determined satisfactorily. Since Bacillus species have only recently been accepted as normal, although infrequent residents of human skin, little information exists on the factors and relationships controlling their survival. In the present study the effects of antibiotic production on the survival of Bacilli and on the composition of normal flora are examined.

Staphylococcus aureus is commonly isolated from eczematous lesions, including atopic dermatitis and dermatophytosis, yet its interactions with other flora and the factors controlling its population have not been elucidated. In a separate investigation, the ecological effect of S. aureus on resident flora of normal and eczematous skin was sought.

These studies support our effort to determine the role of the normal cutaneous organisms in host resistance and susceptibility to common skin infections afflicting soldiers with the objective of developing prophylactic measures against bacteria and fungi.

RESULTS AND DISCUSSION OF THE RESULTS:

Spores of 3 strains of Bacillus licheniformis, including bacitracin-producing ATCC 10716, were applied in sequence to forearms of volunteers in sets of 11. Germination occurred within 24 hours. Dose studies showed that a minimal survival of 2 weeks required an inoculum of $>10^4$ colony-forming units/cm². Variation in the carriage of Bacilli was extreme and individuals could be differentiated into long-term (>21 days) and short-term carriers (<14 days). Compared to control strains, ATCC 10716 survived significantly longer and at higher levels on short-term carriers. At 3 weeks, 45% of subjects supported the Bacillus. The loss of diphtheroids and increase of bacitracin-resistant cocci was observed with carriage of ATCC 10716.

Forty-five paired sites (normal and lesions) were sampled from 37 patients with atopic dermatitis or nummular eczema. In a second survey, 162 sites of a patient with an acute flare of chronic

atopic dermatitis were examined. Normal sites carried fewer but more diverse flora, whereas the composition of flora of lesion sites was restricted mainly to S. aureus or S. epidermidis biotype 1 or both. At densities $>10^5$ cfu/cm², S. aureus seemed to affect surrounding flora; and at densities $>10^6$ cfu/cm², it constituted virtually 100% of the total aerobic bacterial flora.

CONCLUSIONS:

Antibiotic production appears to occur in vivo. Perhaps by eliminating or reducing nearby competitive flora, such activity can prolong the survival of Bacilli on human skin.

S. aureus is such a successful colonizer on eczematous lesions that as it increases in number it also increases in proportion. S. aureus on nearby normal skin are probably contaminants from lesions rather than true residents.

RECOMMENDATIONS:

Survival of Bacilli on regions of the skin with different environments should be tested and interactions among S. aureus and skin flora should be examined quantitatively in vitro.

PUBLICATIONS:

None.

STUDY NO. 3

Interactions of Cutaneous
Microorganisms In Vitro

PROBLEM:

Although in this laboratory, the EcoloGen (New Brunswick Scientific Company) has proven to be a potentially useful tool in investigating microbial interactions, its practicality in supporting in vivo data has not been tested. The study of Bacillus survival on human skin offered an opportunity to examine specific interactions in vitro.

Gram-negative Enterobacteriaceae are frequently isolated from skin subjected to wet occlusion. The genus *Serratia* is characterized by the synthesis of red pigment; however, non-pigmented strains are common. Furthermore, the pigment is lost upon subculturing at 37 C. A simple agar medium was developed to distinguish all *Serratia* from other Enterobacteriaceae.

RESULTS AND DISCUSSION OF THE RESULTS:

Competition was studied by comparing growth curves of representative skin bacteria, including isolates of S. epidermidis biotype 1,

S. saprophyticus biotype 1, M. luteus, and a large-colony diphtheroid, grown individually, in mixed culture, and together in presence of each test strain of B. licheniformis. Diminution of growth of M. luteus and the diphtheroid in the first mixed culture was noted; the diphtheroid was completely inhibited in common culture with Bacillus begin strain ATCC 10716. Lesser antibiotic effects were seen on the cocci, whose rank of sensitivity was similar to that in vivo. The growth of M. luteus and particularly the diphtheroid was enhanced in mixed culture with the non-antibiotic-producing strains of Bacillus.

Over 275 clinical and food isolates and reference strains of Enterobacteriaceae were tested. Included were all major species; 76 isolates were Serratia. Of this family only Serratia were able to hydrolyze Tween 80; this allows the free fatty acid to precipitate with calcium in the agar. An opaque zone around the colony represented a positive reaction.

CONCLUSIONS:

The EcoloGen corroborated in vivo results.

Formerly, Serratia was classified as a member of the Enterobacteriaceae; now Serratia can be differentiated within 24 hours by use of a single agar medium.

RECOMMENDATIONS:

Each test of the EcoloGen required 2 to 3 trials. Because we found that a week was needed for each trial and diffusion through the apparatus was slow, the EcoloGen should be used for only short-term simple studies. A new in vitro apparatus should be devised which will permit rapid diffusion of metabolites yet maintain physical separation of bacteria.

Studies with Serratia should be expanded to substantiate the results; the esterases should be examined.

PUBLICATIONS:

None.

Study No. 4

Alteration of Skin Flora by Soaps

PROBLEM:

Soap would be a simple way to deliver prophylactic antimicrobial agents to the soldier's skin. The term "normal flora" is used frequently in skin microbiology, but with the widespread use of antimicrobial deodorant soap in American military and civilian

populations, it is questionable whether or not the term has relevancy. Most experimental studies empirically have required volunteers to use nondeodorant soaps for 1 to 2 weeks. The validity of this procedure has not been ascertained, and detailed bacteriological analysis of deodorant soap use is limited. The objective of this study was to determine the extent and duration which skin flora is altered by a major commercial deodorant soap.

RESULTS AND DISCUSSION OF THE RESULTS:

Using a cross-over design, 21 volunteers washed their forearms at least once a day with either a trichlorocarbanalide-containing soap or a plain soap for 3 weeks and then switched to another soap for 4-weeks use. No significant difference in total viable counts was noted among individuals, but in 20 subjects an alteration in the composition of skin flora was observed. The deodorant soap tended to reduce or eliminate diphtheroids in 71% of carriers, and fewer kinds of bacteria were also noted. More S. epidermidis was seen with the plain soap, but washing with the deodorant soap seemed to favor Acinetobacter calcoaceticus and Micrococcus luteus.

CONCLUSIONS:

A commercial trichlorocarbanalide-containing soap does not necessarily reduce total counts of bacteria on at least the forearm, but does alter the normal flora. Volunteers should avoid use of such a deodorant soap for a month prior to their participation in experiments.

RECOMMENDATIONS:

Long-term and secondary effects of using deodorant soap should be examined.

PUBLICATIONS:

Bibel D.J. The ecological impact of a commercial deodorant soap on normal skin flora. Abstract. Annual Meeting, Amer. Soc. Microbiol., Atlantic City, 1976.

STUDY NO. 5

Skin Flora Maps

PROBLEM:

In our search for a means to prevent streptococci from colonizing the skin and producing pyoderma and the jungle sore, we found we had no definitive knowledge of the kinds, quantities, and distribution of

bacteria found on the soldier's skin. Under tropical conditions, most soldiers are susceptible to cutaneous streptococcal, while fewer are resistant. Perhaps the resident microflora can aid or prevent streptococci from causing skin disease.

No map depicting an individual's cutaneous flora has been published. Detailed maps can help to determine which sites (if any) are representative for the entire body, what is the degree and consistency of variation among symmetrical sites, if the distribution pattern can be correlated with major anatomical regions, and if characteristic effects occur in subjects with dermatoses. Two investigations were conducted: the first developed mapping as a tool by using subjects with healthy or eczematous skin, and the second examined dynamics of cutaneous microbial populations under the stress of wet occlusion.

RESULTS AND DISCUSSION OF THE RESULTS:

Bacteriological analysis of 162 and 175 skin sites of 2 subjects demonstrated that neither the density nor the kinds of microorganisms were homogeneously arrayed nor were they bilaterally symmetrical. Separate types of flora tended to segregate on large anatomical regions and inhabited overlapping territories. No two sites were exactly alike in their carriage of microorganisms.

Quantitative and qualitative changes in the cutaneous aerobic bacterial flora upon 20 sites on the backs of each of 3 healthy subjects were examined before and after one site was occluded. Major local alterations were found to affect the carriage of microorganisms in distant surrounding areas. S. epidermidis was the most successful competitor. Some sites appeared to act as restricted reservoirs for specific types of microorganisms whereas other areas were less limited in their support of flora.

CONCLUSIONS:

Skin flora maps are a useful tool for studying cutaneous ecology. The removal of wet occlusive dressings can profoundly affect the composition of bacterial flora in distant areas.

RECOMMENDATIONS:

Skin flora maps should be compared with skin maps of lipids, pH, carbon dioxide, and other characteristics to help define habitats and ecological factors.

PUBLICATIONS:

Bibel D.J., and D.J. Lovell: Skin flora maps: a tool in the study of cutaneous ecology. J. Invest. Dermatol. 67:265-269, 1976.

Bibel D.J., D.J. Lovell and R.J. Smiljanic: Effects of occlusion upon population dynamics of skin bacteria. Br. J. Dermatol. (in press)

STUDY NO. 6

Morphological Studies of Dermatophytes and Dermatophytosis

PROBLEM:

Dermatophytic fungi cause severe epidemics of debilitating skin disease among combat troops fighting in tropical areas.

Dermatophytes have been examined by both transmission and scanning electron microscopy. This work has been limited to hyphae, macro- and microconidia, and the mating types. However, the development of these forms has been neglected, and, with the exception of one brief report, arthrospores have not been examined. Now that arthrospores can be produced on agar medium, their development from hyphae can be studied with ease.

RESULTS AND DISCUSSION OF RESULTS:

Microconidia (microaleurospores) of Trichophyton mentagrophytes were plated on Sabouroud's dextrose agar and incubated at 35 C either under normal atmosphere or under 8% CO₂. Formation of multiple rings along the hyphae, often with residual surface matter, ensued. Within the week barrel-shaped spores developed and separated. Transmission electron microscopy did not show surface material or protruding ridges; however, multiple septae and nuclei were observed with hyphae.

CONCLUSIONS:

Classic arthrospore morphology was achieved on agar medium under an atmosphere of 8% CO₂.

RECOMMENDATIONS:

Significance of the surface material of hyphae found under CO₂ should be determined.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6345	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9. LEVEL OF SUM	
76 04 02	H.Termination	U	U	NA	NL	9B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^a				11. TASK AREA NUMBER		12. WORK UNIT NUMBER	
A. PRIMARY				62760A		3A762760A822	
B. CONTRIBUTING				01		074	
C. CONTINUING				CARDS 114(f)			
13. TITLE (Precede with Security Classification Code) ^a							
(U) Nutritional and Metabolic Aspects of Nutrients							
14. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
02300 Biochemistry; 003500 Clinical Medicine							
15. START DATE		16. ESTIMATED COMPLETION DATE		17. FUNDING AGENCY		18. PERFORMANCE METHOD	
66 07		76 09		DA		C. In-House	
19. CONTRACT/GRANT				20. RESOURCES ESTIMATE		21. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				76		3.4	
B. NUMBER: ^a Not Applicable				77		77	
C. TYPE:				77		0.8	
D. AMOUNT:				77		0	
E. KIND OF AWARD:				77		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Biochemistry Division			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Sauberlich, H.E., DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4323			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Joyce, B.E., LTC, MS			
				NAME:			
				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nutrients of Military Rations; (U) Food Preservation							
(U) Nutritional Requirements; (U) Metabolism; (U) Wholesomeness; (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) (a) Provide essential information pertaining to the wholesomeness and nutritional adequacy of foods considered for military ration and feeding systems; (b) establish fundamental information concerning requirements for nutrients and factors that may alter these requirements including various military environments and infectious diseases; (c) study the metabolic interrelationships and functional aspects of nutrients that may influence the physical and mental performance of the individual soldier in any given military situation.</p> <p>24. (U) Studies will involve primarily animal and microbial experimentation for later application to human situations. Isotopically labeled nutrients will be employed to study their metabolism, requirements, interactions, turnover rates, etc., in animals under various controlled dietary and environmental conditions.</p> <p>25. (U) 75 07 - 76 09 Compounds having chromatographic profiles similar to the flavin nucleotides were isolated from tissues of rats fed ¹⁴C-2-riboflavin. Riboflavin itself was the major labeled compound found after incubation of specific crude tissue homogenates. High pressure liquid chromatographic procedures were established for the assay of ascorbic acid (AA), riboflavin, flavin nucleotides and vitamin A. Determination of AA using Fe⁺³/Ferrozine was found to be a rapid and sensitive assay method for this vitamin in plasma, urine and selected food samples. Metabolic activity of the C-6 carbon of AA was examined by two experimental methods and 45% of the C-6 carbon was found to be oxidized <u>in vivo</u>. The precursor of the oxidation may be a slowly exchanging form of AA in which the C-6 carbon is in the primary alcohol oxidation state.</p>							

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*Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	074	Nutritional and Metabolic Aspects of Nutrition

The following investigations have been conducted under this work unit:

STUDY NO. 1	Ascorbic Acid: Chemistry and Biological Functions
STUDY NO. 14	Riboflavin Metabolism in the Rat
STUDY NO. 15	Use of High Pressure Liquid Chromatography in Vitamin Assays

Study No. 1. Studies were conducted on the metabolism of radioactive carbon and tritium labeled vitamin C in macaque monkeys. Results show that ascorbic-6-¹⁴C acid, given intravenously, is not catabolized to ¹⁴CO₂ and the major excretion products are acidic metabolites in the urine. After intravenous dosing with ascorbic-6-³H acid, 43% of the injected tritium was excreted as urinary water. The data indicate that the side chain of ascorbic acid is metabolically active and subject to C-6 oxidation.

Study No. 14. Compounds having chromatographic profiles similar to those shown by the flavin nucleotides were isolated from the tissues of rats administered radioactive carbon labeled riboflavin. Riboflavin itself was the major ¹⁴C labeled compound isolated from crude tissue homogenates.

Study No. 15. High pressure liquid chromatographic procedures were instituted and evaluated for the assay of vitamin A, vitamin C, riboflavin, and the flavin nucleotides. Studies are in progress to determine the optimum conditions for the extraction and purification of these vitamins from biological samples prior to chromatography. The Ferrozine assay has proved to be rapid and sensitive for the detection of ascorbic acid in plasma, urine and selected food samples.

BODY OF REPORT

WORK UNIT NO 074

Nutritional and Metabolic Aspects of
Nutrients

STUDY NO. 1

Ascorbic Acid: Chemistry and Biological
Functions

PROBLEM:

The investigations reported on this work unit were initiated in order to obtain fundamental information concerning the metabolic interrelationships and functional aspects of nutrients that may influence the physical and mental performance of the individual soldier in any given military situation. Previous investigations have shown that the macaque monkey can serve as a good model for studying the metabolism of vitamin C. Metabolic activity of the side chain of ascorbic acid, carbons 5 and 6, usually has not been considered in the ascorbic acid biochemistry of higher animals although several earlier studies have suggested the existence of such a process. The number and diversity of the urinary metabolites found after dosing with radioactive carbon labeled ascorbic acid are difficult to explain without suggesting C-6 oxidation. This study was designed to examine such a process.

RESULTS AND DISCUSSION OF THE RESULTS:

Macaque monkeys weighing about 3 kg were given intravenous doses of ^{14}C -6-ascorbic acid (C-6-AA) and ^3H -6-ascorbic acid (H-6-AA). Breath was monitored for ^{14}C activity. Urine was collected and analyzed for total ^3H and ^{14}C activity and water specific activity; aliquots were subjected to periodate degradation. Release of labeled CO_2 was not seen after intravenous dosing with C-6-AA. Determinations of the urine $^3\text{H}_2\text{O}$ specific activity and organic radioactivity after dosing with H-3-AA yielded parallel, first order curves with a $T_{1/2}$ of 17 to 20 days and the amount of ^3H excreted as water was found to be 43% of the total administered indicating a metabolic process which causes significant release of the C-6 hydrogens. After periodate degradation and isolation and counting of the resulting formaldehyde as the dimedone derivative, 45% of the radioactivity could not be recovered as the formaldehyde derivative. Possible processes that could account for the non-production of formaldehyde include oxidation of the C-6 carbon, derivatization of the C-5 or C-6 hydroxyl groups or reduction of the C-5 or C-6 carbons. Loss of ^3H to water in the tritium experiments clearly shows that the C-6 carbon is being oxidized. This process is of biological interest for at least two reasons. First, C-6 oxidized derivatives of ascorbate have functional groups that allow easy covalent bonding to proteins and polysaccharides in forms that would retain the unique enediol lactone ring and the potential catalytic functions of ascorbate itself.

Second, ascorbic acid and postulated side chain oxidation products are structurally similar to those seen in catecholamine catabolism and these observations lead to questions regarding similarities in the degradative processes and whether or not any intermediates are competitive or interactive.

CONCLUSIONS:

These experiments show that about 45% of the C-6 carbon of ascorbic acid is oxidized in vivo. Lack of CO₂ production indicates that the metabolites do not enter general carbohydrate metabolism. The precursor of the oxidation may be a slowly exchanging form of ascorbic acid in which the C-6 carbon is in the primary alcohol oxidation state.

RECOMMENDATIONS:

These studies should be continued in an attempt to assign chemical structures to the radioactive metabolic products.

PUBLICATIONS:

1. Tolbert, B.M., D.O. Johnsen, B.E. Joyce and E.M. Baker. Nutritional significance and metabolism of ascorbate sulfate. Proceedings of the Xth International Congress of Nutrition, Kyoto, Japan, 3-9 August 1975, p. 14 (Abstract).
2. Halver, J.E., R.R. Smith, B.M. Tolbert and E.M. Baker. Utilization of ascorbic acid in fish. Ann. NY Acad. Sci. 258:81, 1975.
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4. Tolbert, E.M., M. Downing, R.W. Carlson, M.K. Knight and E.M. Baker. Chemistry and metabolism of ascorbic acid and ascorbate sulfate. Ann. NY Acad. Sci. 258:45, 1975.
5. Sauberlich, H.E. Vitamin C status: Methods and findings. Ann. NY Acad. Sci. 258:438, 1975.
6. Joyce, B.E., D.O. Johnsen, R.D. Shlien, B.M. Tolbert and E.M. Baker. Metabolism of ascorbic-6-³H in the monkey. Fed. Proc. 35:660, 1976 (Abstract).
7. Tolbert, B.M., R.J. Harkrader, L.M. Plunkett, H.A. Stuber and D.O. Johnsen. Periodate degradation of ascorbate and ascorbate metabolites. Fed. Proc. 35:661, 1976 (Abstract).
8. Tolbert, B.M., R.J. Harkrader, D.O. Johnsen and B.E. Joyce. C-6 oxidation of ascorbic acid: A major metabolic process in animals. Biochem. Biophys. Res. Comm. 71:1004, 1976.

PROBLEM:

Previous studies on extracts of radioactive labeled riboflavin metabolites from rat tissues suggest that the largest percentage of ^{14}C derived from ^{14}C -2-riboflavin is strongly bound to protein. Isolation of several ^{14}C labeled derivatives of ^{14}C -2-riboflavin from rat urine indicates that riboflavin is metabolized or chemically degraded by the rat. This study was designed to determine the disposition of riboflavin in rat tissue.

RESULTS AND DISCUSSION OF THE RESULTS:

Studies with rats partially depleted of tissue flavins by dietary control confirmed previous observations that 2- ^{14}C -riboflavin was absorbed and retained by the tissues. No $^{14}\text{CO}_2$ was measured during the repletion phase. A minimum of three ^{14}C labeled compounds having chromatographic profiles similar to the flavin nucleotides flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) were isolated from the tissue extracts after chromatography on columns of R-15 resorcinol. The profiles were not altered by acid hydrolysis or incubation in trichloroacetic acid and the compounds were biologically active as determined by microbiological assay. Following incubation of crude tissue homogenates, riboflavin was the major ^{14}C labeled compound isolated from liver, kidney and heart. Homogenates of brain, adrenals and testes were observed to contained ^{14}C labeled compounds that previously have been overlooked. After enzymatic incubation of brain, heart, and adrenal homogenates, only small amounts of riboflavin were isolated. This explains in part why the flavin concentration in specific tissues is not drastically changed during riboflavin depletion.

CONCLUSIONS:

Flavin related compounds other than riboflavin, FMN or FAD are present in rat tissues. The water soluble and biologically active compounds do not appear to be covalently bound flavin peptides. The results suggest that these compounds may not be measured by the routine riboflavin assays used to assess the nutritional status of this vitamin in man.

RECOMMENDATIONS:

Attempts should be made to identify these flavin-related compounds and to establish their roles in riboflavin metabolism in an effort to increase the understanding of riboflavin metabolism in man.

PUBLICATIONS:

Tillotson, J.A. and H.E. Sauberlich. ^{14}C -Riboflavin metabolites in rat tissues. Fed. Proc. 35:660, 1976 (Abstract).

STUDY NO. 15 Use of High Pressure Liquid Chromatography
in Vitamin Assays

PROBLEM:

High pressure liquid chromatography (HPLC) has proved to be a powerful tool for the separation, identification, and quantitation of small quantities of pure vitamins and their metabolites. The major problem lies in extracting these labile compounds in picogram quantities from biological materials and food samples. This study was designed to develop reliable sample handling and extraction procedures so that HPLC could be adopted for routine use in nutrition investigations and military nutrition surveys.

RESULTS AND DISCUSSION OF RESULTS:

A multi-wavelength ultraviolet detector was purchased and evaluated as a means of extending the capability of HPLC for the analysis of picogram quantities of various vitamins. Separation techniques were developed for purified samples of ascorbic acid, riboflavin, the flavin nucleotides and vitamin A. Development of procedures for the extraction of these nutrients from biological and food samples is ongoing. Radioactive ^{14}C derivatives of these compounds are used to verify the specificity of the methods. After extraction and separation of the labeled compounds by HPLC, individual components are collected and the ^{14}C measured to determine if it is localized in only the compound under investigation. The results of these experiments were compared with standard colorimetric methods for the assay of these vitamins and demonstrated the non-specificity of the colorimetric assays. A study was conducted to compare the Fe^{3+} /Ferrozine [3-(2-pyridyl)-5,6-Bis(4-phenylsulfonic acid)-1,2,4-triazine] method for the assay of ascorbic acid and the standard 2,4 dinitrophenylhydrazine (DNP) method with regard to sensitivity and specificity for the determination of this vitamin in biological and food samples. Two hundred selected food samples from a nutrition survey at the Naval Air Station, Alameda, were analyzed by both methods. Interference from other compounds in the samples was minimal with the Ferrozine method but caused problems with the DNP assay.

CONCLUSIONS:

HPLC with multi-wavelength ultraviolet detection provides an excellent means of isolating and quantitating picogram quantities of vitamins in the presence of compounds which interfere with their

measurement by other methods. The Ferrozine assay for ascorbic acid in plasma, urine and selected food samples has proved to be rapid, sensitive and reliable.

RECOMMENDATIONS:

The use of HPLC for the isolation, identification, and quantitation of picogram amounts of nutrients in biological and food samples should be expanded and the Ferrozine assay for ascorbic acid should be adopted for routine use.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OE 6082	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISSEM INSTRN	8b. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H.Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62760A	3A762760A822		00		075	
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code)*							
(U) Environmental and Endocrine Nutrition in Military Personnel							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
002300 Biochemistry; 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 12		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		76	
b. NUMBER: Not Applicable				FISCAL		7T	
c. TYPE:				CURRENT		0	
d. KIND OF AWARD:				77		0	
19. RESPONSIBLE DOD OR ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: Letterman Army Institute of Research Biochemistry Division			
ADDRESS:				ADDRESS: Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Klain, G.J., DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5872			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Sauberlich, H.E., DAC			
				NAME: POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Adaptation; (U) Endocrinology; (U) Environmental Health; (U) Nutrition; (U) Nutrition Disorders; (U) Military Nutrition							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Military personnel under combat and in other stressful environments may have nutritional requirements and nutritional-biochemical characteristics quite different from those of the civilian population. Despite stressful situations, successful completion of military assignments is essential. The objectives of these investigations would be to study (a) the nutritional alterations that may occur with exposure to multiple stresses; (b) the impact of disturbances in endocrine functions and interstresses; (c) the influence of dietary modifications upon resistance to stresses and on effectiveness in performing physical and mental work; and (d) the occurrence of metabolic adaptations to nutritional and dietary alterations and imbalances.</p> <p>24. (U) The objectives will be approached through the study of (1) simultaneous stresses in animals and where possible with human volunteer subjects; (2) responses common to two or more stresses; (3) time sequences of the onset of the responses to particular stresses; and (4) the extent in time of these responses after removal of the measurement of growth and food intake, determination of levels of tissue and urinary metabolites, radiochemical studies, determination of metabolic pathways, clinical observations, and measurement of physical and mental performance.</p> <p>25. (U) 75 07 - 76 09 Inhibition of hepatic fatty acid synthesis by glucagon is less severe in fasted-refed rats than in fed controls. This effect is accompanied by a marked reduction of acetyl CoA carboxylase and fatty acid synthetase activities. In contrast, the hormone does not affect adipose tissue lipogenesis. The hepatic effects of glucagon are readily reversed by exogenous insulin. In addition, glucagon depresses hepatic synthesis of cholesterol.</p>							

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	075	Environmental and Endocrine Nutrition in Military Personnel

The following investigation has been conducted under this work unit during the past year:

STUDY NO. 2 Nutritional State and Glucagon Effects on Fatty Acid Synthesis

STUDY NO. 3 In Vivo Effects of Glucagon on Hepatic Synthesis of Cholesterol

Studies No. 2 and 3. In vivo effects of glucagon and insulin on several aspects of fatty acid synthesis were studied in fed, fasted, and fasted-refed rats. The results indicate that glucagon depressed hepatic fatty acid synthesis and glucose oxidation in fed and fasted-refed rats. However, this effect was less pronounced in fasted-refed rats than in the fed rats. In contrast, fatty acid synthesis in fasted animals was not reduced by glucagon below the level associated with fasting per se. Furthermore, glucagon markedly reduced the activity of hepatic acetyl CoA carboxylase and fatty acid synthetase. Exogenous insulin antagonized the glucagon effects and restored hepatic lipogenesis in fed or fasted-refed animals. In addition, glucagon reduced hepatic synthesis of cholesterol and fatty acid synthesis in the heart and the kidney. In contrast, the hormone had no effect on adipose tissue lipogenesis.

BODY OF REPORT

WORK UNIT NO. 075

Environmental and Endocrine Nutrition
in Military Personnel

STUDY NO. 2

Nutritional State and Glucagon Effects
on Fatty Acids Synthesis

PROBLEM:

Alterations in the nutritional state of an animal often times evoke compensatory changes in systemic levels of several hormones which in turn drastically alter a number of metabolic parameters. Fasting, as an extreme nutritional state, enhances plasma levels of several catabolic hormones including glucagon and reduces the level of insulin. In contrast, hyperalimentation associated with refeeding after a period of food deprivation reverses the plasma levels of the two hormones. Since glucagon and insulin in vivo are simultaneously transported to the liver from the pancreas via the portal vein, it has been suggested that the molar ratio of glucagon:insulin determines the catabolic or anabolic state of the organism, i.e., the rise in the glucagon:insulin ratio observed during fasting favors catabolic processes. Associated with these are reduced activities of the key lipogenic enzymes, and enhanced activities of gluconeogenic and of several amino acid degrading enzymes. A decline in the glucagon:insulin ratio, as a result of refeeding, promotes anabolic processes of all types.

In this respect, it has been demonstrated that in vitro reduction of hepatic fatty acid synthesis by glucagon is less severe in rats refed after fasting than in ad libitum fed animals. Such observations strongly suggest that endogenous levels of insulin in refed rats partially antagonize inhibitory effects of glucagon on fatty acid synthesis. Available data further show that fatty acid synthesis in fasted rats is not reduced by glucagon below the level associated with fasting per se. In view of these observations, the present study was conducted to determine in vivo effects of glucagon and insulin on various aspects of lipid synthesis from ^{14}C -glucose in fed, fasted or refed rats. The importance of glucagon in the moment-to-moment regulation of nutrient homeostasis has been recognized; its role in mitigating harmful effects of stress as may be encountered in military combat is thus apparent.

RESULTS AND DISCUSSION OF THE RESULTS:

Male rats ranging in weight from 300 to 350 g were fed a complete casein-sucrose diet for ten days. After this period of dietary adjustment, the rats were randomly divided into three treatment groups. The first group continued on the ad libitum feeding schedule while the second and the third group of animals were fasted for three days. The

third group of animals was subsequently refed for three days. Thereafter, the animals were injected intravenously with either glucagon (1 mg/kg body weight), insulin (0.5 unit/kg body weight) or a combination of the two hormones. Twenty minutes later the animals were sacrificed and glucose-U- ^{14}C incorporation into hepatic and adipose tissue fatty acids and $^{14}\text{CO}_2$ was determined. In addition, the activity of acetyl CoA carboxylase and fatty acid synthetase in the two tissues was assayed.

Compared to the controls, glucagon reduced hepatic glucose oxidation, fatty acid synthesis, and the activity of acetyl CoA carboxylase and fatty acid synthetase in fed or refed rats. No glucagon effect was observed in fasted animals. In addition, glucagon did not affect fatty acid synthesis or enzyme activity in any of the three experimental groups. Insulin stimulated glucose conversion into hepatic fatty acids in fed animals but had no effect on fatty acid synthesis in refed animals. Insulin restored fatty acids synthesis and enzyme activity that had been inhibited by glucagon in both fed and refed animals. No insulin effect was observed in fasted animals.

CONCLUSIONS:

The present study demonstrates pronounced effects of altered nutritional states on hepatic responses to glucagon and to insulin. Inhibition of hepatic fatty acids synthesis by glucagon can be readily overcome by exogenous insulin. Glucagon has no effect on fatty acid synthesis in adipose tissue. Consequently, it appears that fatty acids synthesis in adipose tissue may be controlled by mechanisms different from those found in hepatic tissue.

RECOMMENDATIONS:

Further investigations of the basic interrelationships between glucagon and insulin and glucose metabolism are needed. These should include studies concerned with interactions between the two hormones and various nutritional states.

STUDY NO. 3

In Vivo Effects of Glucagon on Hepatic
Cholesterol Synthesis

PROBLEM:

It has been reported that in vivo hepatic synthesis of cholesterol is stimulated, reduced, or unchanged after administration of glucagon. It appears that the results of such studies would depend on the nutritional state of the experimental animal since glucagon effects in vivo may be antagonized by other hormones, such as insulin. Furthermore, the dose and the method of administration of the hormone would also affect experimental results. Accordingly, in the following study, in vivo effects of glucagon on hepatic cholesterol synthesis from

acetate in fed rats were investigated. In addition, acetate incorporation into fatty acids in various tissues was determined.

RESULTS AND DISCUSSION OF THE RESULTS:

Fed rats were injected intravenously with glucagon and five minutes later the animals were injected with a saline solution of sodium acetate-1-¹⁴C (8.0 μ Ci/100 g body weight). Immediately thereafter, each animal was placed in a glass metabolism cage and expired ¹⁴CO₂ was collected for 20 minutes in an aqueous solution of 2% NaOH. Thereafter the animals were decapitated and liver, kidney, heart, epididymal fat pads and gastrocnemius muscle were removed and rinsed in ice-cold saline. Total lipids were extracted, saponified, and radioactivity in fatty acids was determined. Ten ml of 1% digitonin in 90% ethanol were added to a portion of the hepatic total lipid extract and cholesterol-digitonide was allowed to precipitate overnight at 0 C. The precipitate was then washed with ethanol, acetone, and anhydrous ethyl ether. A portion of the dried precipitate was weighed and dissolved in Hyamine at 60 C and radioactivity was determined as indicated above.

Compared to the control animals, glucagon had no effect on in vivo acetate oxidation. The hormone, however, reduced in vivo acetate incorporation by approximately 33, 30 and 77%, respectively, into liver, kidney and heart fatty acids but had no effect on fatty acid synthesis in adipose tissue or skeletal muscle. Moreover, the hormone depressed synthesis of hepatic cholesterol by about 33%.

CONCLUSIONS:

Intravenous administration of glucagon depressed synthesis of hepatic cholesterol. In addition, the hormone reduced incorporation of acetate into hepatic, kidney, and heart fatty acids.

RECOMMENDATIONS:

Additional studies should be conducted to delineate the effects of glucagon on enzyme systems involved in the synthesis of cholesterol. In addition, studies concerned with the effects of glucagon on cholesterol turnover are recommended.

PUBLICATIONS:

1. Hecker, A.L., G.J. Klain, E.W. Askew, and W.R. Wise, Jr. Adaption to exercise: leucine metabolism in skeletal muscle. *Physiologist* 18:242, 1975 (Abstract).
2. Klain, G.J., A.W. Meikle and T.P. O'Barr. Gluconeogenic response to mannoheptulose in the rat. *J. Nutr.* 106:68, 1976.

3. Klain, G.J. Glucagon and glucose utilization in fed, fasted and fasted-refed rats. Fed. Proc. 35:363, 1976 (Abstract).
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5. Klain, G.J. and J.P. Hannon. Kidney response to cold stress and high protein intake. Proc. Soc. Exp. Biol. Med. 153:393, 1976.
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7. Klain, G.J. Hepatic utilization of glutamate during the hibernation cycle. Life Sci. 19:591, 1976.
8. Klain, G.J. In vivo lysine oxidation and incorporation into tissue proteins by fasted-refed rats. Nutr. Rep. Int., 1976 (In press).
9. Klain, G.J., F.J. Sullivan, K.S.K. Chinn, J.P. Hannon and L.D. Jones. Metabolic responses to prolonged fasting and subsequent re-feeding in the pig. J. Nutr. (In press).
10. Klain, G.J. In vivo effects of glucagon on fatty acid synthesis in fasted and refed rats. J. Nutr. (In press).
11. Charles, M.A., J. Lawecki, A.L. Steiner, and G.M. Grodsky. Adenosine 3',5'-monophosphate in pancreatic islets: Tolbutamide- and arginine-induced insulin release. Diabetes 25:256, 1976.
12. Gerich, J.E., M.A. Charles, and G.M. Grodsky. Regulation of pancreatic insulin and glucagon secretion. Annu. Rev. Physiol. 38:353, 1976.
13. Charles, M.A., W. Imagawa, P.H. Forsham, and G.M. Grodsky. Islet transplantation into rat liver: In vivo secretion of insulin from the isolated perfused liver and in vivo glucagon suppression. Endocrinology 98:738, 1976.
14. Gerich, J.E., M.A. Charles, and G.M. Grodsky. Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. J. Clin. Invest. 54:833, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTRN	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3M762772A811		00	003		
b. XXXXXXXXXX	62760A	3A762760A822		01	076		
c. XXXXXXXXXX	CARDS 114f						
11. TITLE (Precede with Security Classification Code)*							
(U) Analytical Biochemistry							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
002300 Biochemistry; 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		4.3	
b. NUMBER* Not Applicable				FISCAL		1.0	
c. TYPE:				YEAR		60	
e. KIND OF AWARD:				CURRENT		4.0	
f. CUM. AMT.				77		139	
19. RESPONSIBLE DOD OF SANITIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS*				NAME* Letterman Army Institute of Research Biochemistry Division Department of Nutrition Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME* Skala, J.H., DAC TELEPHONE: 415-561-5872 SOCIAL SECURITY ACCOUNT NUMBER:			
RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415-561-3600				ASSOCIATE INVESTIGATORS NAME: Sauberlich, H.E., DAC			
21. GENERAL USE				POC:DA			
Foreign Intelligence Not Applicable							
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Analytical Biochemistry; (U) Instrumentation; (U) Automated Analyses; (U) Nutrition Surveys; (U) Clinical Chemistry; (U) Mil Medicine							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Develop and adapt new concepts in analytical biochemistry to provide reliable and advanced procedures and services to military-oriented research programs of all departments, LAIR, and on occasion to approved cooperating agencies; to innovate or develop analytical procedures to meet specific needs of such research as, for example, the development of micro-automated assay procedures for enzymes related or altered during nutritional deficiencies, disease states, or stress conditions. Develop procedures applicable to military nutrition surveys, ration test studies and food wholesomeness evaluations.</p> <p>24. (U) Analytical support will be provided to studies in military medicine requiring routine analyses in volume or unique equipment and special techniques for assays of physiological specimens in the evaluation of the nutritional requirements and dietary adequacy of military personnel. Specific analyses will be originated or adapted as required to meet the needs of specific studies and to improve the economy and efficiency of laboratory operations. Research will be conducted on a continuing basis in support of the objectives indicated to provide new methods and, whenever feasible and practical, automated and computer linked.</p> <p>25. (U) 75 07 - 76 09 Analytical support requiring more than 20,000 analyses was provided to 27 research projects from five departments. Methods were developed for determining transketolase and glutathione reductase activities and stimulation effects in erythrocytes on a centrifugal fast analyzer. An automated amino analyzer was acquired to meet the requirements for present and anticipated analyses being generated by Institute wide research.</p>							

*Available to contractors upon originator's approval.

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	076	Analytical Biochemistry

The following investigations have been conducted under this work unit:

STUDY NO. 1	Analytical Support and Services
STUDY NO. 2	Development of Analytical Biochemistry Procedures

Study No. 1. Analytical support requiring 23,253 individual analyses was provided to 27 research projects.

Study No. 2. Methods for determining activities and stimulation effects for transketolase, glutathione reductase, and glutamic-oxalacetic transaminase in erythrocytes were developed for the centrifugal analyzer. Methods for cholesterol and triglycerides were implemented with slight modification for use on this analyzer; a method for methylmalonic acid was implemented with slight modification for a continuous flow analyzer. Improvements were made in the functionality and safe operation of the high vacuum components in the mass spectrometry sample inlet and preparation systems.

BODY OF REPORT

WORK UNIT NO. 076

Analytical Biochemistry

STUDY NO. 1

Analytical Support and Services

PROBLEM:

Chemical analysis of various physiological specimens and diet or ration items is a fundamental adjunct to the majority of the research objectives of the many research protocols active at LAIR. The Analytical Biochemistry Branch has the responsibilities for providing service to the investigating staff in the form of automated analytical support.

RESULTS AND DISCUSSION OF THE RESULTS:

The Branch provided support to 27 research projects, including two field studies, which resulted in a total of 23,253 automated, semi-automated, and manual analyses. These analyses were distributed by type as follows.

<u>Analytical Service</u>	<u>Number of Analyses</u>
Blood Chemistry:	
(1) Automated electrolytes, glucose, total protein, urea nitrogen, creatinine, iron, cholesterol, triglycerides, vitamin C, lactate, pyruvate, uric acid, hemoglobin, and various enzymes	11,457
(2) Semi-automated lipid phosphorus, osmolality, total iron binding capacity, and non-esterified fatty acids	2,554
(3) Manual alpha amino nitrogen, hematocrit, and electrophoresis	916
Urine Chemistry:	
(1) Automated electrolytes, creatinine, urea nitrogen, iron, and uric acid	5,606
(2) Semi-automated osmolality, and oxalate	248
(3) Manual ammonia, titrable acidity, specific gravity, pH, catecholamines, and screens	588

Food Chemistry:

(1) Semi-automated bomb calorimetry	332
(2) Manual proximate analyses, fatty acid gas chromatography profiles	1,552

The total number of analyses was distributed among the various departments as follows: Nutrition, 7855 (33.8%); Surgery, 6087 (26.2%); Medicine, 2121 (9.1%); Comparative Medicine, 150 (0.6%); and Biomedical Stress, 120 (0.5%). The remainder of the analytical commitment (6920 (29.8%)) was to field study support (Work Unit 086, Nutrition Studies in Support of DOD Food Program).

CONCLUSIONS:

Although operating at 90% of its previous staffing level, the Branch had sufficient personnel and equipment to support all requests for analyses which were or could be automated. The automated analysis load increased approximately 69% (corrected for the 15-month reporting period) over the previous year, while there was a 56% increase in semi-automated and manual analyses. A backlog exists in the area involving diet, ration items, urine and stool analyses, which require semi-automated or manual procedures.

The alleviation of the backlog is hampered because of the following deficiencies:

a. The Branch has not had functioning perchloric acid hood for 30 months. This has delayed the digestion of various specimens for minerals and total nitrogen.

b. Bench and hood space is insufficient for the manual sample handling necessary for semi-automated or manual analyses. The Branch must relocate solvent extraction systems to a hood. At the present time, hoods available to the Branch are not large enough to accommodate the systems. Since there is no space to install larger hoods without sacrificing bench space, it has been suggested that these systems be relocated to a solvent laboratory area. Then the additional bench space could be used by the staff to perform manual diet and ration analyses. It is anticipated that these analyses could be performed more efficiently and that the backlog could be reduced.

RECOMMENDATIONS:

Chemical analysis of physiological and food specimens is an essential component of mission-oriented research at the laboratory. It is recommended that the Analytical Biochemistry Branch continue to function as a central resource for such support. Principal efforts

PUBLICATIONS:

None.

PROBLEM:

RESULTS AND DISCUSSION OF THE RESULTS:

Enzymatic methods for serum total cholesterol and triglycerides have been implemented on a centrifugal analyzer. A method is being investigated for determining food cholesterol using a proprietary compound to form a water soluble derivative with subsequent analysis by the enzymatic centrifugal analyzer procedure. Centrifugal analyzer methods were developed for the determination of baseline and cofactor stimulated activities of transketolase, glutathione reductase, and glutamic-oxalacetic transaminase (aspartate aminotransferase) in erythrocytes.

The efficacy of the transketolase assays was evaluated in a study with thiamin deficient rats.

A semi-automated procedure for determining methylmalonic acid in urine was evaluated, slightly modified, and implemented for support of a metabolic study.

In cooperation with another branch, which performs microbiological vitamin assays, a program for least squares curve fitting and concentration calculation was developed for use on a Branch computer with hardcopy output. The procedure saves the analyst considerable computation time.

A chemist with high vacuum and cryogenic experience was hired at the beginning of the fiscal year and was trained on the isotope ratio mass spectrometer. Several improvements were made in the high vacuum glass sample preparation and sample inlet systems. The dual carbon dioxide preparation lines have been modified so that the Toepler pumps can be isolated from the rest of the system during sample introduction. The control of these pumps has been altered so that one pump is slave to the other providing synchronous and safer operation. The sample inlet system on the mass spectrometer has been modified by installing a roughing manifold to separate the high and low vacuum areas. The glass components and valves have been modified so that less than 1 cc of noncondensable gas can be handled with negligible loss between the sample tube and the molecular leak.

CONCLUSIONS:

The Branch is equipped to provide support in automated analyses of clinical and nutritional specimens and in the more sophisticated areas of amino acid studies and stable isotope use. The centrifugal analyzer has proven capable both in end-point and kinetic chemistries.

RECOMMENDATIONS:

Continued review of operations is recommended with the objective of automating and improving procedures.

PUBLICATIONS:

Knight, M.K., P.P. Waring and J.H. Skala. Assessment of thiamin status of rats using erythrocyte transketolase activity. Fed. Proc. 35:443, 1976 (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 75 07 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8A. DISB'N INSTR'N NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A811		00		005	
b. SECONDARY	62760A	3A762760A822		01		079	
c. THIRDARY	CARDS 114F						
11. TITLE (Precede with Security Classification Code)* (U) Radioisotope Support for Military Medical Research							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 008500 Isotopes; 013900 Radioactivity; 011000 Nuclear Instrumentation							
13. START DATE 64 05		14. ESTIMATED COMPLETION DATE CONT		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE*		EXPIRATION:		PRECEDENCE		b. FUNDS (In thousands)	
b. NUMBER* Not Applicable				76		0.7	
c. TYPE:		d. AMOUNT:		FISCAL YEAR		7T	
				CURRENT		0.3	
e. KIND OF AWARD:		f. CUM. AMT.		77		1.2	
						49	
19. RESPONSIBLE DOD ORGANIZATION NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS* RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415-561-3600				20. PERFORMING ORGANIZATION NAME* Letterman Army Institute of Research Radioisotope Division ADDRESS* Department of Nutrition Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME* Morrissey, R.L., CPT, VC TELEPHONE: 415-561-4770 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: NAME:			
21. GENERAL USE Foreign Intelligence Not Applicable				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Research Projects; (U) Radioisotopes; (U) Instrumentation; (U) Data Acquisition							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) To provide radioisotope support to all projects requiring the use of radioisotopes and improve procedures and counting techniques where needed. Conduct radioactive counting procedures for cooperating joint military medical research projects. To conduct research to improve technology and to adapt existing technology to research areas of significance to the LAIR mission. 24. (U) Methodology research is conducted as required to improve existing procedures. Nine automatic sample changing radiation detection instruments are maintained for detection of beta and gamma radiation. All aspects of the radiological protection program as required by Nuclear Regulatory Commission licensure are conducted. 25. (U) 75 07 - 76 09 Research support has been provided to 34 radioisotope investigators during this period. Support provided includes procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, radioactive waste disposal and maintenance of logs and records as required by Nuclear Regulatory Commission and Army regulations. Consultation concerning proper use and application of radioisotope technology has been provided as needed. Through the cooperative effort of the HPO, LAMC, The Health Physicist, LAMC and the RPO, LAIR, a 32 hour training course entitled "Safe Use and Handling of Radioisotopes" was presented.							

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	079	Radioisotope Support for Military Medical Research

Research investigators are currently being supported with radioisotope services, including procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, waste disposal, maintenance of appropriate logs and records, and maintenance of radiation detection instruments for investigator use. A 32-hour training course entitled "The Safe Use and Handling of Radioisotopes" was presented during the current fiscal year.

BODY OF REPORT

WORK UNIT NO. 079

Radioisotope Support for Military
Medical Research

PROBLEM:

The use of radioisotopes in biomedical research has proven to be useful. The Radioisotope Division is responsible for procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, radioactive waste disposal and maintenance of logs and records as required by Nuclear Regulatory Commission (NRC) and Army regulations. Advice and counsel are given to investigators regarding the use of radioisotopes. Beta and Gamma counting instruments are maintained for use of investigators at LAIR in support of military medical research.

RESULTS AND DISCUSSION OF RESULTS:

Support functions delegated to the Radioisotope Division were maintained in the current fiscal year. Current instrumentation includes 4 gamma counting instruments and 7 liquid scintillation counters. When the Department of Information Sciences is able to provide the computer programming support such as we previously had at the USAMRNL in Denver, we will be able to provide the services of disintegrations per minute determinations and additional mathematical calculations.

Bulk procurement of radioisotope support supplies such as liquid scintillation counting solutions and vials results in price difference savings. Additional savings are accrued by processing a few large orders instead of numerous small orders from the 34 research investigators currently authorized to use radioisotopes.

Through the cooperative effort of the Health Physics Officer, LAMC, the Health Physicist, LAMC, and the Radiation Protection Officer, LAIR, a 32-hour training course entitled "Safe Use and Handling of Radioisotopes" was presented.

The radioisotope support facility was moved from LAIR Phase I to LAIR Phase II during the current fiscal year. This move permitted expansion and isolation of the gamma counting facility.

CONCLUSIONS AND RECOMMENDATIONS:

The use of radioisotopes is essential to the mission of LAIR. It is recommended that the centralized support activity be maintained as the most economical and efficient means of making radioisotopes available to research investigators while maintaining adequate control of their use and thus protecting the health of laboratory personnel. Technological studies should continue to

identify the most efficient, the most accurate, and the most economical laboratory procedures to be used in the routine use of radioisotopes.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DAOA 6364	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTRN*	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D Change	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3S762772A814				025	
B. CONTRACTOR	62760A	3A762760A822		01		082	
C. CONTRACTOR	CARDS 114f						
11. TITLE (Precede with Security Classification Code)*							
(U) Mathematical and Computer Support of Military Biomedical Research							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
09700 Mathematical and Statistics; 002300 Biochemistry; 021900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 02		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				B. FISCAL YEAR		D. FUNDS (In thousands)	
B. NUMBER: Not Applicable				76		3.0	
C. TYPE:				77		0.8	
D. KIND OF AWARD:				77		98	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: Letterman Army Institute of Research			
ADDRESS:				ADDRESS: Department of Information Sciences Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Chastain, R. V. Jr., CPT, MS			
TELEPHONE: (415)561-3600				TELEPHONE: (415) 561-5541			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence not applicable				ASSOCIATE INVESTIGATORS			
				NAME: Waibel, K. J. CPT, MS; Gums, James W.			
				NAME: DAC; Hopkins, L. Alan, DAC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Mathematics; (U) Statistics; (U) Research Data;							
(U) Processing and Analysis; (U) Support of Military Biomedical Research							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
(U) To provide mathematical, statistical and computer support for military biomedical research at LAIR.							
24. (U) Consultation is provided in the appropriate and efficient use of available statistical and mathematical programs. Generally, studies are planned to provide valid and thorough statistical analysis for a variety of biomedical research needs. Studies emphasize making analysis readily available to and interpretable by researchers without a statistical or computer background. In-house data processing is planned and distributed on a basis of need. These systems permit investigators to automatically acquire data, and to conduct analyses interactively.							
25. (U) 75 07 - 76 09. Two new packages of statistical routines are now being maintained. These packages provide statistical capabilities not previously available at LAIR. In addition, special applications programs were provided, and consultations given in experimental design and statistical analysis. The nucleus of an in-house distributed resource processing system was installed and is being readied for use.							

*Available to contractors upon originator's approval.

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	082	Mathematical and Computer Support of Military Biomedical Research

The following investigations have been conducted under this work unit:

- STUDY NO. 1. Computerized Mathematical and Statistical Operations
- STUDY NO. 2. Mathematical and Statistical Analysis Training Manual
- STUDY NO. 3. Design of Distributed Information Processing Facilities

To provide LAIR researchers with an expanding repertoire of statistical routines, two new program packages are now available. Biomedical Computer Programs (BMDP) contain a collection of statistical programs designed specifically for biological and medical applications. Another package consists of programs which evaluate nonlinear mathematical models. Also applications programs have been written to aid researchers in specific data analysis problems. Computer and non-computer support has been given in areas of experimental design as well as data analyses. An in-house distributed computer system has been procured. The major components of this system were received during the reporting period.

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT TECHNICAL REPORT.(U)
SEP 76

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BODY OF REPORT

WORK UNIT NO. 082

Mathematical and Computer Support
of Military Biomedical Research

STUDY NO. 1

Computerized Mathematical and
Statistical Applications

PROBLEM:

Statistics is a collection of methods which allows one to make objective evaluations of uncertain inferences. Certain aspects of statistics are valuable in evaluating biomedical phenomena. Knowledge of valid statistical methods may enable an investigator to (1) formulate testable hypotheses, (2) plan an experiment efficiently so that sufficient data are provided to test hypotheses, but time and resources will not be spent collecting extraneous data, and (3) analyze data so that hypotheses are evaluated accurately. Knowledge of statistical methods permit an investigator to estimate certain characteristics (parameters) of a population or populations. In particular, sampling theory enables an investigator to collect samples which are representative of the population(s) under study. Theories of point and interval estimation provide a standard and consistent approach to the problem of making inferences from samples about the magnitude or location of parameters in the population.

To perform statistical analyses of the magnitude required by the Military by hand is time-consuming and impractical. Our objective is to use computer capabilities to their maximum to develop a variety of general programs which can be readily adapted to a variety of requirements introduced by LAIR's investigators and to project future military requirements for the computer in biomedical investigations. There are four primary program packages used for statistical analysis at LAIR. BMDP, SPSS, GRASS and IMSL provide a wide range of statistical computing capabilities. Software for analysis of specialized problems are available via NONLIN or special programs written in the Department of Information Sciences.

RESULTS AND DISCUSSION OF THE RESULTS:

The Generalized Research Analysis Statistical System (GRASS) is maintained at Lawrence Berkeley Laboratory (LBL) by the Department of Information Sciences to support LAIR investigators. GRASS has the capability to produce a variety of descriptive statistics, plots, histograms, and non-parametric tests. GRASS also has a variety of data transformation and data manipulation capabilities. Furthermore, a new collection of programs, called NONLIN, has been implemented to compute nonlinear regression analyses to fit nonlinear theoretical models to experimental data.

The Statistical Package for the Social Sciences (SPSS), an integrated system of computer programs for data manipulation and analysis, and International Mathematical and Statistical Library (IMSL) which contains over 185 subroutines around which a user may build his own special purpose programs, are also available at LBL for LAIR users.

Three applications programs were written to aid LAIR researchers with specific analyses not available through packaged programs. All three programs concern problems in analysis of variance by using repeated measures. One program provides a generalized repeated measure ANOVA for experiments with unequal group sizes while another provides an orthogonal decomposition of variance to allow for trend analysis. The third program tests for compound symmetry of covariance matrices. This procedure is useful for testing the mathematical assumption in a repeated measures ANOVA model and provides a test of equality of covariance matrices useful in multivariate analysis.

CONCLUSION:

This study provides an expanding repertoire of computational routines to assist all LAIR researchers in collecting and analyzing their data. Computerized statistical analyses minimize errors and make possible a variety of analyses which would be impracticable to perform by hand. Furthermore, an investigator may select which statistical techniques are most appropriate to employ without having to master the mechanics of the techniques.

RECOMMENDATIONS:

Subsets of the major statistical packages mentioned should be implemented on the new in-house minicomputer to allow easier access and faster processing of data for LAIR's investigators. BMDP and SPSS should be updated as new and expanded versions become available.

STUDY NO. 2

Mathematical and Statistical Analysis
Consultation

PROBLEM:

Since efficiency in data collection and analysis is gained by the careful planning of experiments, and familiarity with appropriate statistical principles is required to comprehend many of the problems of experimental design, it is desirable for investigators to have some exposure to basic statistical principles and techniques. The purpose of this study is to provide the exposure for LAIR investigators so that they will not only create an experimental design unique to the military, but can derive maximum benefits for the military from their data.

RESULTS AND DISCUSSION OF RESULTS:

Consultation is available on an as-requested basis.

RECOMMENDATIONS:

A series of sessions should be conducted so that researchers will have a forum in which to discuss statistical problems arising from their own research.

STUDY NO. 3

Design of Distributed Information
Processing Facilities

PROBLEM:

Biomedical engineering support requirements will increase as the level of experiment sophistication rises. The objective of this study is to maintain a responsive state of the art automatic data processing system of hardware and software capable of supporting the biomedical research activities at Letterman Army Institute of Research.

RESULTS AND DISCUSSION OF THE RESULTS:

a. Hardware and Facilities:

1. LBL Computer Services. The CDC 6600/7600 Computer System located at Lawrence Berkeley Laboratory (LBL) Computer Center, Berkeley, California, provides basic general computer support via the CDC 200 User Terminal.

2. MODCOMP II. The MODCOMP II minicomputer system is being developed for the Bioenergetics Division Treadmill Automated System (TAS). Additional core memory for the computer is being procured to provide sufficient space for the executive operating system, the TAS software, and the application programs.

3. Data General NOVA 3/12. The NOVA 3/12 minicomputer with 32K words of memory, dual floppy disk, teleprinter, graphics display tube, 16 channels of analog to digital, and two channels of digital to analog has been procured for the Combat Surgery Division. This equipment is portable and will be used in ophthalmologic, thoracic, pulmonary, physiologic, and anesthesiologic research.

4. Data General ECLIPSE C/300. This recently procured computer system consists of 128K words of memory, two 90 million character disk drives, a 300-line/minute card reader, two cathode ray tube terminals, a magnetic tape drive, equipment for 24 communication lines within LAIR and two outside lines.

5. Proposed Data General NOVA 3/12. This minicomputer has been proposed for the Military Performance Division who provides general support in experimental psychology on an interdepartmental and inter-agency need basis. It will specifically support neurobehavioral investigations and stress analysis.

b. Software Development:

1. MODCOMP II. The Treadmill Automated System (TAS) software is currently awaiting the installation of additional memory on the MODCOMP computer for final checkout. Upon successful checkout, the TAS system will be ready for use.

2. Data General NOVA 3/12. The system software is being implemented.

3. Data General ECLIPSE C/300. The system software checkout is awaiting final completion of the hardware installation. After it is completed, data base, statistical and program development will be the major uses of the computer.

CONCLUSION:

During the reported period significant enhancements to the LAIR data processing resources were accomplished. These enhancements will make data processing and problem solving power directly accessible to the users and programmers alike. Researchers will have the capability to solve spur-of-the-moment or one-time problems and get their results promptly. Use of interactive computing by researchers throughout LAIR relieves the programming staff of work which users can do for themselves, and greatly reduces the cost of developing small scale or occasional use programs.

RECOMMENDATIONS:

The distributed processing should be continued and expansion should be driven by the demands of the investigator's requirements. Persons responsible for planning and budgeting at LAIR must continue to be cognizant of the implications of computer support systems for military biomedical research. They must program funds far enough in advance so that as the level of experiment sophistication rises, systems and biomedical engineering support requirements can be supported through the purchase of additional equipment.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISB'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H.Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A822	01	085			
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Nutritional Requirements of Military Personnel							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
00800 Agr. Economics; 002300 Biochemistry; 003500 Clinical Medicine							
13. START DATE	14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD		
71 07	76 09		DA		C. In-House		
17. CONTRACT/GRANT			18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS		b. FUNDS (In thousands)
a. DATES/EFFECTIVE:			PRECEDING		3.2		120
b. NUMBER: ^a Not Applicable			76		0.8		50
c. TYPE:			FISCAL				
d. AMOUNT:			77		0		0
e. KIND OF AWARD:			CURRENCY				
19. RESPONSIBLE DOD ORGANIZATION			20. PERFORMING ORGANIZATION				
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129			NAME: ^a Letterman Army Institute of Research Biochemistry Division				
ADDRESS: ^a			ADDRESS: ^a Department of Nutrition Presidio of San Francisco, CA 94129				
RESPONSIBLE INDIVIDUAL			PRINCIPAL INVESTIGATOR (Furnish SCAN II U.S. Academic Institution)				
NAME: Canham, J.E., COL, MC			NAME: ^a Sauberlich, H.E., DAC				
TELEPHONE: 415-561-3600			TELEPHONE: 415-561-4323				
21. GENERAL USE			SOCIAL SECURITY ACCOUNT NUMBER:				
Foreign Intelligence Not Applicable			ASSOCIATE INVESTIGATORS				
			NAME: Joyce, B.E., LTC, MS				
			NAME:				
			POC:DA				
22. KEYWORDS (Precede EACH with Security Classification Code) ^a (U) Military Rations and Feeding; (U) Human Micro and Macro Nutrient Requirements; (U) Military Nutrition Surveys; (U) Laboratory Animals							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To evaluate military rations, existent and experimental, in terms of chemical composition and macro- and micro nutrient content; to study the effect of such rations on the nutrient status of military personnel; to define the requirements for micro-nutrients in military situations and various environs and provide guidance for ration formulation on this basis; to investigate the parameters of nutrient status for various micro-nutrients to enable the early recognition of nutritional insufficiencies or excesses; to develop biochemical techniques to support these investigations and facilitate measurement of nutritional status.							
24. (U) A search for new urinary and blood parameters useful in defining nutritional status and the development of analytical methods to use these parameters will be accomplished. Following preliminary animal studies, volunteer human subjects will be studied under strict metabolic ward observation or during military field studies to permit more accurate definition of nutrient requirements under various conditions or environments common to the military.							
25. (U) 75 07 - 76 09 Modules were designed and constructed for analysis of erythrocyte transketolase (ETK), transaminase (EGOT), and glutathione reductase (EGR) using the Autoanalyzer II. Animal and human samples were evaluated as to sensitivity, range, optimum conditions and applicability to assess B-vitamin status. Data indicate that the Autoanalyzer II methods for ETK and EGOT can be adopted for routine use while that for EGR is unsatisfactory. Adaptation of a continuous flow method for the assay of ascorbic acid was completed. Commercial kits for radioassay of serum folate were evaluated for use with whole blood hemolysates. None have proved reliable to date and work is in progress to develop a suitable radioassay for this vitamin in hemolysates. A computer program was developed to give final concentration values for the microbiological assay of B vitamins.							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	085	Nutritional Requirements of Military Personnel

The following investigations have been conducted under this work unit:

STUDY NO. 1 Vitamin C Metabolism and Requirement in Man

STUDY NO. 4 Evaluation of Analytical Procedures Utilized in
Military Nutrition Surveys

Study No. 1. A method for continuous flow measurement of serum ascorbic acid utilizing the AutoAnalyzer II was developed, evaluated, and adopted for routine use for vitamin C analysis of serum samples.

Study No. 4. Studies were conducted on the application of new or improved enzymatic, microbiological and radioimmune assays (RIA) for the measurement of vitamin B₆, vitamin B₁₂, folic acid, riboflavin, and thiamin.

BODY OF REPORT

WORK UNIT NO. 085

Nutritional Requirements of Military Personnel

STUDY NO. 1

Vitamin C Metabolism and Requirement in Man

PROBLEM:

Vitamin C nutritional status may be determined through clinical examinations or biochemical measurements. Clinical signs are not reliable indicators of nutritional status; therefore, for the most part, biochemical measurements represent the most objective assessment of the vitamin C status of an individual. The determination of the serum level of vitamin C has been the most commonly used biochemical measurement. However, the methods employed are tedious and lack sensitivity. Investigations were initiated to develop an improved procedure.

RESULTS AND DISCUSSION OF THE RESULTS:

An automated, continuous flow method utilizing the AutoAnalyzer II and the manual 2,6-dichloroindophenol dye method formed the basis for development of the analytical system. Various modifications and improvements were implemented to meet particular needs. Ascorbic acid in serum or plasma was stabilized with metaphosphoric acid (HPO_3) immediately after the sample was separated from the cells. (This provides a protein-free filtrate that inhibits the catalytic oxidation of ascorbic acid by Fe^{3+} and Cu^{2+} and may be stored frozen for several weeks.) Elimination of a dialysis step in the analytical scheme enhanced the sensitivity of the assay while allowing small volumes of samples to be used. Interferences from indophenol reducers (sulfhydryl compounds, sulfites, and thiosulfates) and phenolic compounds were minimized by keeping the pH of the reaction at about 3.5. The stability of ascorbic acid in fresh serum and frozen serum, with or without HPO_3 treatment, was studied. Compared with the initial values for the fresh serum, values for untreated frozen serum decreased by 10-15% within 24 hours and by 30% at the end of 6 days, while the frozen HPO_3 -treated serum had a mean loss of about 3% during a 13-day storage period. The coefficient of correlation for ascorbic acid standard curves covering the range of 0.5 to 5.0 mg/liter and at a sampling rate of 60/h was 0.9956; the standard error of the estimate was equivalent to 0.17 mg of ascorbic acid per liter. This method measures only the reduced form of ascorbic acid. However, dehydroascorbic acid, the oxidized form, is not present in significant amounts in properly prepared and preserved serum samples. The 2,4-dinitrophenylhydrazine method measures both ascorbic acid and dehydroascorbic acid. However, for use with serum samples, the procedure is tedious, requires long incubation periods and strong acid and is less satisfactory for adaptation to an automated system.

CONCLUSIONS:

This automated, continuous flow procedure has proved to be a sensitive, rapid and reproducible method for the measurement of ascorbic acid in serum. With properly prepared and preserved samples, it provides values which correlate well with those obtained by standard, 2,4-dinitrophenylhydrazine methods.

RECOMMENDATIONS:

This method should be adopted for routine use for vitamin C analysis of a large number of serum samples.

PUBLICATIONS:

Sauberlich, H.E., W.C. Goad, J.H. Skala and P.P. Waring. Procedure for mechanized (continuous-flow) measurement of serum ascorbic acid (vitamin C). Clin. Chem. 22:105, 1976.

STUDY NO. 4 Evaluation of Analytical Procedures Utilized
in Military Nutrition Surveys

PROBLEM:

Needs exist for rapid procedures which are accurate, reproducible and practical for assessing vitamin nutriture. Manual microbiological or enzymatic methods are used to measure vitamin B₆, folic acid, vitamin B₁₂, thiamin, riboflavin, and other vitamins. The procedures are tedious and often do not provide the desired precision and reproducibility. Endeavors were made to develop new or improved methods for assessing vitamin nutriture of military personnel or for measuring the vitamin content of military rations.

RESULTS AND DISCUSSION OF RESULTS:

Design and construction of a module for the continuous flow analysis of erythrocyte transketolase (ETK) activity as a means of assessing thiamin status was completed. A preliminary study in rats was made to establish the sensitivity, range, and applicability of the method for thiamin deficient animals. Erythrocyte preparations from human subjects were analyzed to establish normal range, storage effects and optimum reaction conditions. Preliminary data indicate that assessing thiamin status by stimulation of ETK by thiamin pyrophosphate (TPP) was less indicative of thiamin deficiency than was measurement of ETK activity itself and may not reflect true status if used exclusively.

An AutoAnalyzer II (AA II) module for analysis of erythrocyte transaminase activity as a means of assessing vitamin B₆ nutriture was designed and evaluated to establish normal range, sensitivity, and

optimum reaction conditions with hemolysates from human subjects. Further evaluation of the data awaits completion of a rat study with vitamin B₆ deficient animals. Analytical methods for measuring glutathione reductase activity by continuous flow and centrifugal fast analyzer (GEMSAEC) procedures were compared. Results indicate that analysis on the GEMSAEC is acceptable for use in assessing riboflavin status while analysis on the AA II is unsatisfactory at this time.

Several commercial kits for the radioassay of folic acid were evaluated. Although these are satisfactory for serum folate assay, none has proved reliable for use with whole blood hemolysates. Work is currently in progress to develop a suitable radioassay for folic acid in hemolysates. A computer program for a least squares solution of a curvilinear equation (standard curve) was written to calculate final concentration values from raw microbiological vitamin assay data for plasma, urine, and food samples. This has eliminated the time-consuming method of reading values from a non-linear standard curve which was subject to human error.

CONCLUSIONS:

Progress was made on new or improved enzymatic, microbiological, and radioassay methods for assessing B-vitamin nutriture.

RECOMMENDATIONS:

None.

PUBLICATIONS:

1. Knight, M.K., P.P. Waring and J.H. Skala. Assessment of thiamin status of rats using erythrocyte transketolase activity. Fed. Proc. 35:443, 1976.
2. Sauberlich, H.E. Functional and biochemical tests for the assessment of vitamin nutritional status. In: Proceedings of Western Hemisphere Nutritional Congress IV, edited by P.L. White & N. Selvy; Acton, MA: Publishing Sciences Group, Inc., 1975, p. 185.
3. Sauberlich, H.E. Vitamin metabolism and requirements, some aspects reviewed. S. Afr. Med. J., 49:2235, 1975.
4. Hodges, R.E., H.E. Sauberlich, J.E. Canham, L. Mejia, C. Lykke and D.L. Wallace. Iron deficiency anemia and hypovitaminosis. Proceedings of the Xth International Congress of Nutrition, Kyoto, Japan, 3-9 Aug 75, p. 176.
5. Sauberlich, H.E. Excerpts from the Xth International Congress of Nutrition Meeting. Am. J. Clin. Nutr. 29:226, 1976.

6. Hodges, R.E., H.E. Sauberlich, J.E. Canham, D.L. Wallace, R.B. Rucker, L.A. Mejia, and M. Mohanram. Hematopoietic studies in vitamin A deficiency. (Submitted for publication)

7. Vitamin A deficiency and xerophthalmia. Report of a Joint WHO/USAID Meeting. Technical Report Series No. 590, Geneva, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 75 07 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8A. DISB'N INSTR'M NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT	
10. NO. / CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	62772A	3M762772A810			003			
B. XXXXXXXXXX	62760A	3A762760A822		01	155			
C. XXXXXXXXXX	CARDS 114 (f)							
11. TITLE (Precede with Security Classification Code)* (U) More Effective Topical Repellents Against Malaria-Bearing Mosquitoes								
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 - Clinical Medicine								
13. START DATE 67 11		14. ESTIMATED COMPLETION DATE CONT		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House		
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS		B. FUNDS (In thousands)
A. DATES/EFFECTIVE:				PRECEDING 76		2.4		30
D. NUMBER*				FISCAL 77		.6		3
C. TYPE: Not Applicable				CURRENT 77		2.4		69
E. KIND OF AWARD:								
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION				
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research				
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Department of Dermatology Research Presidio of San Francisco, CA 94129				
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)				
NAME: Canham, J.E., COL, MC				NAME* Spencer, T.S., CPT, MS				
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5485				
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:				
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS				
				NAME: Akers, W.A., COL, MC				
				NAME: POC: DA				
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Topical Repellents; (U) Human Volunteers; (U) Insect Repellent; (U) Tropical Diseases; (U) Mosquito; (U) Skin; (U) Stratum Corneum; (U) Polymer Formulations								
23. TECHNICAL OBJECTIVE* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.) 23. (U) To discover a long-lasting, water and abrasion resistant, topical repellent formulation which will protect soldiers against malaria-bearing mosquitoes and other vectors of militarily important diseases. To develop <u>in vitro</u> test methods to determine physical properties of repellent formulations which are tested in the <u>in vivo</u> screening program. By correlation of <u>in vitro</u> and <u>in vivo</u> test results, to predict evaporation, penetration, and repellent-skin interactions, aiding in the design of new repellents.								
24. (U) To continue repellent evaluation in coordination with the Department of Tropical Medicine. To determine permeability of skin to candidate repellents <u>in vitro</u> and relate results to repellent-skin interactions <u>in vivo</u> . Insufficient number of subjects for studies of human factors in repellency may impede progress. To develop a computer base for statistical analysis of data on repellents; the analysis will determine which individual characteristics can be enhanced to promote longer duration of repellent protection.								
25. (U) 75 07 - 76 09 Fatty acids found on the skin surface were isolated and correlated with attractiveness to mosquitoes and repellent protection times against mosquitoes. Repellent volatilities were measured for 8 repellents and correlated with repellent efficacy <u>in vivo</u> . After experimentation with several membranes, a model system was developed to measure evaporation and penetration through skin. A computer data management system was loaded with data from mosquito repellent test, enabling investigators to access information concerning protection times and physical properties of repellents.								

*Available to contractors upon originator's approval.

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A76270A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	155	More Effective Topical Repellents Against Malaria Bearing Mosquitoes

The following investigations have been conducted under this work unit:

- STUDY NO. 5 Data Management System for Mosquito Repellent Data
- STUDY NO. 6 Repellent Efficacy and Composition of Skin Lipids
- STUDY NO. 8 Methodology for Determination of Repellent Volatility In Vitro
- STUDY NO. 9 Repellent Loss from Skin In Vitro: Effect of Different Membranes on Repellent Evaporation and Penetration

Studies No. 5, 6, 8 and 9 Certain fatty acids which are found on the skin surface were isolated and correlated with attractiveness to mosquitoes and repellent protection time against mosquitoes. Repellent volatilities were measured for 8 repellents and correlated with repellent efficacy in vivo. Several membranes were tested in an in vitro system to measure repellent evaporation and penetration, and a model system was constructed with whole skin to measure simultaneous evaporation and penetration through skin. A computer data management system adequately stored data collected on mosquito repellent tests. Although the retrieval system was effective, data had to be reloaded into a second program for statistical analysis.

BODY OF REPORT

WORK UNIT NO. 155

More Effective Topical Repellents
Against Malaria-Bearing Mosquitos

STUDY NO. 5

Data Management System for Mosquito
Repellent Data

PROBLEM:

A large volume of data concerning repellent efficacy on humans and physical properties of repellents has been collected over a six-year period. To analyze these data, a computer retrieval system was established as described in the 1975 annual report.

RESULTS AND DISCUSSION OF THE RESULTS:

In FY 76 and 77, repellent, volunteer, and test data covering the initial six years of the mosquito repellent project were coded and loaded into the Remote File Management System (RFMS). Data from this system were used to establish particular individual mean repellent protection times for specific repellents.

The means and standard deviations for each individual confirmed previous observations that there is a large biological difference in repellent protection times afforded different individuals, but each individual has a relatively consistent protection time relative to the group. These means were used (1) to establish correlations between lipid extract data and repellent protection times against mosquitoes and (2) to place certain individuals in a profile continuum of repellent protection effectiveness. Routine retrievals were made on repellent protection times determining overall average effectiveness for 5 repellents over a series of tests. Although the retrieval system was effective, statistical analysis and cross-correlation of data could be accomplished only by reloading the data into a statistical program.

CONCLUSION

The RFMS has the ability to store data which can be retrieved effectively; the system has limited statistical analysis options.

RECOMMENDATIONS:

The data now stored in the RFMS system should be transferred to the Statistical Package for Social Sciences (SPSS) system. The SPSS data management system not only stores data and conditionally retrieves information but is linked to a statistical system which could correlate specified human and repellent factors with repellent

effectiveness. For the purpose of long-term data analysis, future data obtained from new volunteers and repellents should be added to the SPSS storage system as it is accumulated.

STUDY NO. 6

Repellent Efficacy and Composition
of Skin Lipids

PROBLEM:

As a part of a program to develop a repellent formulation to protect troops from vector-borne disease, a mosquito repellent testing program has been carried out over a period of years. Certain individuals in the testing program had consistently longer repellent protection times against mosquitoes than average; conversely, other individuals had much shorter repellent protection times. It was hypothesized that components on the skin surface were important in distinguishing between the two groups. Skin extracts were collected by acetone washes from volunteer's elbows according to the methodology described in the 1975 annual report.

RESULTS AND DISCUSSION OF THE RESULTS:

Subsequent to the preliminary analysis of data reported in the 1975 Annual Report, saturated fatty acids C_7 to C_{18} and unsaturated fatty acids C_{14} to C_{18} were analyzed quantitatively for each volunteer. Multiple regression analysis was used to identify important fatty acids contributing to duration of repellent protection against mosquitoes and to volunteer mosquito attractiveness. The presence of higher quantities of saturated fatty acids C_7 , C_{12} , C_{16} , C_{17} and C_{18} was related to prolonged repellent protection times. The saturated fatty acid C_{14} and unsaturated fatty acid C_{15} influenced mosquito attractiveness. Saturated C_{11} was important in both cases, it had a positive effect on repellent protection time and a negative effect on attractiveness, i.e. repellency constitutes negative attractiveness. A positive correlation between the total lipid content and repellent protection time for each individual was related to previous work which indicates that certain additives can prolong repellent duration on the skin surface.

CONCLUSION:

Certain acetone extraction components, in a small population, had significant effects on repellent protection time and mosquito attractiveness.

RECOMMENDATIONS:

Skin lipid content should be analyzed for a larger population of volunteers (1) to establish if the designated fatty acids are consistently important to repellent protection time and mosquito

attractiveness and (2) to determine if the naturally occurring skin lipids can be used to induce longer repellent protection against mosquitoes.

STUDY NO. 8

Methodology for Determination of
Repellent Volatility In Vitro

PROBLEM:

As a part of a program to develop a more effective mosquito repellent to protect troops from vector-borne disease, repellent volatility is one factor that should be considered. Repellent volatility is important in any screening program where repellent compounds are being systematically synthesized and tested for biological activity against mosquitoes.

RECOMMENDATIONS:

Repellent volatility were measured with a Dupont 990 Thermal Evolution Analyzer. Experimental repellent compounds carbamide, sulfonamide, SRI-6, SRI-7, SRI-8, and SRI-9 had volatilities 2 to 5 times lower than the standard military repellent deet. In addition to having lower volatility than deet, carbamide, sulfonamide, and SRI-7, appeared to persist on the skin longer and appeared to afford increased repellent protection time against mosquitoes. Since there are several other factors which may influence repellent duration, including skin permeability, trans-epidermal water loss, individual attractiveness to mosquitoes, or intrinsic repellency of the repellent molecule, the relation between repellent volatility and repellent duration can be used only as a preliminary indicator of efficacy and needs to be considered along with other factors. Repellent volatility can be used for the preliminary estimation of the minimum effective dose of a repellent that repels mosquitoes. The minimum effective dose of repellent increases as the volatility of that repellent decreases; that is, the amount of repellent reaching the sensory apparatus of the mosquito is a function of the volatility of the repellent and the amount applied. Thus, if two chemical structures are being compared for intrinsic repellency of the repellent molecule, the minimum number of repellent molecules necessary to repel a mosquito will be a function of the minimum applied dose which will repel mosquitoes and the volatility of that repellent compound.

CONCLUSIONS:

Repellent volatility is one factor to be considered in any repellent screening program where repellent compounds are being systematically synthesized and tested for biological activity against mosquitoes.

RECOMMENDATIONS:

Repellent volatility should be instituted as a routine measurement for all biologically active repellents studied in a repellent screening program.

PUBLICATIONS:

1. Gabel, M. L., T. S. Spencer, and W. A. Akers: Evaporation rates and protection times of repellents. Mosq. News, J. Am. Mosq. Contr. Assn., 36:141-146, 1976.
2. Spencer, T. S. and W. A. Akers: Field trials of mosquito repellents in Florida, California and Alaska. Proceedings of California Mosq. Contr. Assn., 44:102-105, 1976.

PROBLEM:

In the process of developing a longer-lasting repellent formulation, to protect troops from vector borne disease, numerous compounds and formulations are evaluated for persistence on the skin surface and for rate of repellent evaporation (a minimum rate of evaporation is necessary to repel mosquitoes). Since testing large numbers of formulations against mosquitoes is time-consuming and does not provide physical data on relative evaporation and penetration through skin, an in vitro model is desirable for evaluating and developing longer-lasting repellent formulations.

RESULTS AND DISCUSSION OF RESULTS:

The evaporation rate of the standard military repellent deet from a scratched aluminum planchet was similar to the evaporation of deet from dry stratum corneum; moreover, the persistence of a 1 mg/cm² dose was similar in each case. Both evaporation rate and persistence were reduced in a partially hydrated stratum corneum; presumably, this was the result of penetration. To develop the penetration loss more completely, Ringers lactate solution was used to wet the stratum corneum from below. The evaporation rate varied greatly from sample to sample; and a high proportion of the applied repellent evaporated from the surface. Subsequently, when whole skin was used, the sample-to-sample variability decreased while the relative proportion of repellent lost by evaporation was reduced. For whole skin, 40 to 60% evaporated while 60 to 40% penetrated and, for stratum corneum, 70 to 80% evaporated while 20 to 30% penetrated. Moreover, the persistence of repellent on the surface of whole skin was similar to the effective duration of protection for the repellent deet when tested in vivo against mosquitoes. A boudruche membrane (cattle intestinal membrane) was also tested for comparison to the whole skin; however, most of the applied dose penetrated the membrane rapidly.

CONCLUSION:

Whole skin affords a better model for evaporation-penetration studies of repellent than separated stratum corneum or intestinal membrane.

RECOMMENDATION:

A model system of whole skin should be developed for future studies of repellents and repellent formulations.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OE 6080	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTR*N	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A811				010	
b. XXXXXXXXXX	62760A	3A762760A822		01		156	
c. XXXXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code)* (U) The Metabolic Response of Hepatic and Extra-Hepatic Tissues to Dietary Substances, Drugs and Hormones in Health and Disease.							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 12		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER: Not Applicable				76		4	
c. TYPE:				FISCAL		1	
e. KIND OF AWARD:				7T		33	
f. CUM. AMT.				YEAR		4	
77						102	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Herman, R. H., COL, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME: POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Hepatic Disease; (U) Hepatic Enzymes; (U) Enzyme Deficiency Disease, (U) Adaptive Responses of Hepatic Enzymes; (U) Laboratory Animals (U) Human Volunteers.							
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.) 23.(U) Military personnel require highly responsive adaptive mechanisms for the regulation of blood glucose and other substances which are essential for normal mental and muscular activity. Previous studies have shown that certain hepatic enzymes adapt (i.e., increase or decrease in activity) to diet, folic acid, hormones and medications. These adaptive responses maintain the blood glucose level by coordinating the processes of gluconeogenesis, glycolysis, glycogenesis, glycogenolysis, lipogenesis and lipolysis depending on the physiological state. In combat, impairment of these complex adaptations may interfere with performance during training and combat operations and during recovery from injury. 24.(U) The hepatic adaptive responses of selected patients with hypoglycemia and other disorders of blood glucose regulation and related problems will be studied. The effect of diet, folic acid and certain therapeutic agents on blood glucose regulation will be studied. The acute effect of hormones on hepatic enzymes will be tested on animals. 25.(U) 75 06-76 09 Hydroxocobalamin, a potent form of vitamin B ₁₂ alleviated the subjective symptoms in patients with painful diabetic neuropathy but did not affect methylmalonic acid excretion suggesting that one of the vitamin B ₁₂ cofactors is involved in the utilization of glucose by nervous tissue. We tested the acute effect of gastrin, cholecystokinin (CCK), secretin, glucagon, histamine and serotonin on hepatic enzyme activities and cyclic nucleotides. Glucagon increased and serotonin and secretin decreased blood glucose. Glucagon increased gluconeogenetic enzyme activities and cyclic-AMP while secretin decreased pyruvate kinase (PK) activity and cyclic-AMP. CCK, histamine, serotonin and gastrin increased PK activity. Serotonin increased cyclic-AMP levels and decreased cyclic-GMP levels. In one of three patients with hypoglycemia, hyperinsulinemia was found after glucose ingestion but not after intravenous glucose.							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT	156	Metabolic Response of Hepatic and Extra-Hepatic Tissues to Dietary Substances, Drugs and Hormones in Health and Disease

The following investigations have been conducted under this work unit:

- STUDY NO. 1. The Study of Patients with Diabetic Neuropathy.
- STUDY NO. 2a. Effects of Intravenous Gastrointestinal Hormones on Plasma Glucose and Hepatic Gluconeogenic and Glycolytic Enzyme Activities and Cyclic AMP and GMP Levels in the Rat.
- STUDY NO. 2b. Effects of Intravenous Amino Acids on Plasma Glucose and Hepatic Gluconeogenic and Glycolytic Enzyme Activities in the Rat.
- STUDY NO. 3. Studies on the Regulation and Production of Fuel.
- STUDY NO. 5. Studies of a Patient with Hypertension.
- STUDY NO. 6. The Study of a Patient with Idiopathic Bone Disease Resembling Stress Fractures.

Study No. 1. There are some data to show that vitamin B₁₂ may be useful in treating diabetic neuropathy particularly when intractable pain is present. Recently hydroxocobalamin (a metabolic derivative of vitamin B₁₂ (cyanocobalamin)) has given encouraging results in some patients with painful diabetic neuropathy. Based on the hypothesis that hydroxocobalamin is transformed into coenzyme B₁₂ which is a cofactor in the reaction which transforms methylmalonyl CoA into succinyl CoA, a study was instituted to determine the effect of hydroxocobalamin on methylmalonic acid excretion after the administration of sodium propionate or valine. If methylmalonyl CoA accumulates because of a defect in vitamin B₁₂ metabolism then methylmalonyl CoA perhaps may be incorporated into nerve cell membranes (as has been demonstrated in pernicious anemia) and give rise to the neuropathic state. A more easily metabolized form of vitamin B₁₂, viz., hydroxocobalamin, may enhance the removal of methylmalonyl CoA thereby decreasing the excretion of methylmalonic acid and alleviating the neuropathic symptoms. Three patients with painful diabetic neuropathy

were studied. All three patients had subjective improvement in their pain. In one patient, objective evidence of improvement in sensory discrimination was noted. No reproducible pattern of methylmalonic acid excretion was seen. The period of follow-up is insufficient to assess the changes in nerve conduction velocity or to warrant conclusions about the ultimate benefit of hydroxocobalmin treatment.

Study No. 2a. Glucagon and insulin acutely alter the activity of hepatic glycolytic and gluconeogenic enzymes. These enzymes are involved in the regulation of blood glucose levels under various physiological conditions of feeding, fasting, and exercise and in pathological conditions of injury and infection. We tested the effect of other gastrointestinal (GI) hormones including glucagon, secretin, gastrin, cholecystokinin (CCK), histamine and serotonin on hepatic glycolytic and gluconeogenic enzymes, cyclic-nucleotides and blood glucose levels. Glucagon increased plasma glucose levels while serotonin and secretin decreased plasma glucose levels. Glucagon increased fructosediphosphatase (FDPase), pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) activities and decreased pyruvate kinase activity. Secretin decreased FDPase, PC and PK activities, while CCK and gastrin increased PK activity. Histamine and serotonin increased PK and PC and decreased FDPase activities. Histamine increased PEPCK activity. Glucagon and serotonin increased cyclic-AMP levels while secretin decreased cyclic-AMP. Serotonin increased cyclic-GMP levels. These studies indicate an interrelationship between gastrointestinal hormones and liver enzyme activity and may provide an explanation how enteral feeding can result in regulation of blood glucose via release of GI hormones which then affect the critical enzymes involved in glycolysis and gluconeogenesis.

Study No. 2b. In conjunction with Study No. 2a, the acute effect of various amino acids on hepatic enzyme activities was studied. We tested the effect of leucine, valine, alanine, arginine and tryptophan on blood glucose and hepatic glycolytic and gluconeogenic enzymes. Alanine increased and leucine and arginine decreased blood glucose levels. Arginine increased PC and PEPCK activities, alanine increased PC and FDPase activities and decreased PK, and leucine decreased FDPase activity. These results indicate that amino acids derived from nutrients can directly affect hepatic enzyme activities acutely.

Study No. 3. Studies in a military population have suggested that as many as 23% of male recruits may have "asymptomatic" hypoglycemia following oral ingestion of glucose while at rest. Since glucose utilization continues at an exaggerated rate during exercise, it is possible that the same group might experience symptomatic hypoglycemia during exercise. Hypoglycemia can lead to diminished mental and physical performance. Studies were performed in 3 patients with symptomatic hypoglycemia and in one patient with a suspected defect in folic acid metabolism. In one patient, abnormally high

levels of insulin were found during rest and exercise after a glucose load. Since intravenous glucose did not cause similar insulin release, it is suspected that some unknown gastrointestinal factor may be responsible. Further studies are needed to clarify the mechanisms whereby reactive hypoglycemia is produced. Additional studies of gastrointestinal factors and insulin-like substances during rest and exercise should be done in both normal subjects and patients with reactive and other types of hypoglycemia.

Study No. 5. Essential hypertension is a disease affecting approximately 10% of the population. The cause of essential hypertension is unknown, but some cases may be due to disorders of hormone regulation. Some of the hormones that affect blood pressure also are integrally involved in regulating glucose metabolism. At the request of physicians from Letterman Army Medical Center, a young Army nurse with severe essential hypertension, unresponsive to conventional therapy, was studied. No abnormality in glucocorticoids or mineralocorticoids was found. Such studies should be provided as a service to military patients and their physicians.

Study No. 6. Spontaneous (march or stress) fractures are a cause of significant morbidity to soldiers in training, maneuvers, and combat. It is possible that a subtle form of bone disease predisposes some soldiers to such injury. Vitamin D plays a key role in bone formation, remodeling, and adaptation to stress. A patient with stress fractures was examined for a disorder in calcium and vitamin D metabolism. No obvious defects in vitamin D metabolism were found but an adrenal adenoma producing excess cortisol was found. This patient had bone disease due to Cushing's syndrome that mimicked stress fracture. Further studies should be done to assess the role of adrenocortical steroids in vitamin D metabolism. It is planned to do such studies in work unit 070.

BODY OF REPORT

WORK UNIT NO. 156

Metabolic Response of Hepatic and
Extra-Hepatic Tissues to Dietary
Substances and Hormones in
Health and Disease

STUDY NO. 1.

The Study of Patients with
Diabetic Neuropathy

PROBLEM:

The maintenance of blood glucose levels and the proper production and utilization of other fuel substrates (amino acids and fatty acids) are vital in order for a soldier to function efficiently. Failure to maintain the blood glucose results in decreased mental and physical performance during both rest and exercise. The regulation of endogenous fuel production for utilization by muscle and brain is a complex system involving the interaction of various hormones and enzymes that affect liver, adipose tissue and muscle. During exercise there is production of lactic acid, glycerol, free fatty acids and amino acids which are transported to the liver where the process of gluconeogenesis results in production of glucose that can be used to provide energy. A similar gluconeogenetic mechanism is operative during the early phases of trauma and recovery.

In order to understand the mechanisms involved in the gluconeogenetic states of exercise or injury it is useful to study patients with naturally occurring disorders of gluconeogenesis. Inadequate gluconeogenesis following a glucose load can result in reactive hypoglycemia (see Study No. 3). Diabetes mellitus (D.M.) is a common disorder characterized by excessive gluconeogenesis and ketogenesis due to the relative or absolute lack of insulin and the increased activity of glucagon and epinephrine. An understanding of the mechanisms involved in patients with altered gluconeogenesis can be applied toward the understanding of gluconeogenesis during exercise and trauma.

The neuropathy that occurs in D.M. is a puzzling complication. It may occur with mild D.M. or precede the onset of the disease. It is generally accepted, although not invariably true, that good control of D.M. can improve the neuropathy. Why neurological dysfunction should occur as a result of uncontrolled gluconeogenesis is an enigma.

Several studies have suggested that treatment with vitamin B₁₂ can improve the neuropathy associated with D.M.

Lack of vitamin B₁₂ can lead to subacute combined degeneration, a disease which in some ways resembles diabetic neuropathy. There

is no obvious relationships between vitamin B₁₂ and diabetic neuropathy. However, vitamin B₁₂ is an obligatory cofactor in the enzymatic reaction that converts L-methylmalonyl CoA to succinyl CoA. In a patient with a deficiency of the enzyme necessary for that conversion, a neurological defect was noted. It was postulated that accumulation of methylmalonyl CoA and propionyl CoA permitted abnormal fatty acid synthesis, since each could replace malonyl CoA and acetyl CoA, respectively. The result would be the formation of branched- or odd-chain fatty acids which would be incorporated in the myelin sheath and lead to defective nerve transmission. Analysis of nerve tissue from a patient with the enzyme deficit showed that odd-chain and branched fatty acids were present. In a similar disorder affecting children (propionic-acidemia) there is accumulation of odd-chain fatty acids and neurological deficits occur. Deficiency of B₁₂ has been shown to lead to accumulation of the same type of abnormal fatty acids in nerves with subsequent development of neuropathy. With the observation that B₁₂ may be beneficial in diabetic neuropathy, it is possible to postulate that a defect similar to that seen in methylmalonic aciduria, propionicaciduria and subacute combined degeneration may occur. Vitamin B₁₂ may lead to increased activity of the enzyme for which it is a cofactor or there may be some defect in the conversion of B₁₂ to hydroxocobalamin, its active metabolite.

This study was designed to test the possibility that a defect in the propionic acid pathway may be involved in the neuropathy associated with diabetes mellitus.

RESULTS AND DISCUSSION OF THE RESULTS:

Three insulin-dependent patients with diabetes mellitus have been studied. The patients were well-controlled with diet and insulin. The response of urinary methylmalonic acid to sodium propionate (two patients) or valine (one patient) loading was studied before and during therapy with hydroxocobalamin. Serial neurological examinations were conducted at frequent intervals during the study. Nerve conduction studies were performed before and during hydroxocobalamin therapy. Evaluation of the long-term effects of hydroxocobalamin therapy has not been possible. Short term evaluation has shown subjective improvement in the pain in all 3 patients. Nerve conduction velocity was improved in 2 patients after one month of therapy. Sensory discrimination, tested in only one patient using a Von Frey aesthesiometer, was improved after 3 weeks of treatment. No reproducible changes were noted in urinary methylmalonic acid content after valine or propionate loading before or during B₁₂ treatment.

It is possible that hydroxocobalamin therapy was beneficial. It is also possible that increased attention to diet and diabetes control explains the symptomatic improvement, however, a short trial with similar attention to diet and diabetic control but placebo injections did not produce improvement in one patient who subsequently reported improvement with hydroxocobalamin treatment.

CONCLUSIONS:

Our results to date are encouraging in that they suggest that diabetic neuropathy may be improved by treatment with hydroxocobalamin. Because of the small numbers of patients, we have not been able to correlate urinary excretion of methylmalonic acid with the severity of the disease or improvement following treatment.

RECOMMENDATIONS:

Further studies are needed to determine if therapy with hydroxocobalamin will produce both short- and long-term improvement in diabetic neuropathy. Larger numbers of patients will be required to determine if methylmalonic aciduria correlates with the disease or the therapy. It would then be desirable to investigate the occurrence of methylmalonic aciduria in traumatized patients or normal subjects during prolonged exercise with loading doses of valine or propionate and with or without hydroxocobalamin therapy.

PUBLICATIONS: None.

STUDY NO. 2a.

Effects of Intravenous Gastro-intestinal Hormones on Plasma Glucose and Hepatic Gluconeogenic and Glycolytic Enzymes Activities and Cyclic AMP and GMP Levels in the Rat.

PROBLEM:

Glucagon and insulin have been shown to regulate acutely certain enzymes involved in hepatic glycolysis and gluconeogenesis. These hormones appear to act in reciprocal fashion both with regard to regulation of blood sugar and activation and inactivation of certain hepatic enzymes (phosphofructokinase, pyruvate kinase (PK) and fructosediphosphatase (FDPase)). Both the regulation of blood glucose and alterations in these enzyme activities occur within minutes following the intravenous injection of these hormones. Many of the gastrointestinal (GI) hormones (such as secretin, cholecystokinin-pancreozymin (CCK-PZ), gastrin) stimulate pancreatic release of insulin and to a lesser extent glucagon. To date, no one has studied the effects of these GI hormones on the activation or inactivation of hepatic glycolytic and gluconeogenic enzymes and cyclic nucleotides (cAMP and cGMP).

It is known that oral and intravenous glucose produce different effects on blood sugar levels. Oral glucose may precipitate mild hypoglycemia while intravenous glucose does not. These observations strongly suggest that the oral administration of glucose causes the release

of certain GI hormones (or other substances) which may act on the pancreas to stimulate insulin release. Since many of the GI hormones produce just such an effect, we designed these studies to answer several questions. (1) Does the intravenous administration of individual GI hormones produce significant changes in blood glucose levels, hepatic glycolytic and gluconeogenic enzymes activities and hepatic cAMP and cGMP levels? (2) Do these changes in the biochemical parameters (if any) correlate with the acute changes in blood glucose? (3) Are any of the acute effects of these GI hormones mediated by either cAMP or cGMP? (4) Are changes in blood glucose levels correlated with changes in the ratios of plasma insulin and glucagon?

RESULTS AND DISCUSSION OF RESULTS:

Of the GI hormones tested only glucagon produced a significant increase in plasma glucose levels (62 mg% and 91 mg%, respectively, at 11 and 21 minutes post-injection). Two GI hormones (serotonin and secretin) significantly decreased plasma glucose levels 45 mg% and 25 mg%, respectively. Histamine, CCK-PZ and gastrin had no significant effects on plasma glucose levels.

Changes in hepatic gluconeogenic and glycolytic enzyme activities following intravenous injection of the GI hormones did not, in general, correlate well with changes in plasma glucose levels. The one exception was glucagon which significantly increased the three gluconeogenic enzymes measured (FDPase 16%; pyruvate carboxylase (PC) 38% and phosphoenolpyruvate carboxykinase (PEPCK) 25%) and significantly decreased hepatic PK activity by 46%. Secretin significantly decreased hepatic FDPase (27%), PC (39%) and PK (27%), but had no significant effect on PEPCK activity. CCK-PZ and gastrin significantly increased hepatic PK activity 141% and 66%, respectively, but had no significant effects upon the other enzymes. Histamine and serotonin significantly increased hepatic PK (113% and 78%, respectively) and PC (27% and 69%, respectively) activities and decreased hepatic FDPase activity (40% and 37%, respectively). Histamine significantly increased hepatic PEPCK activity (22%), while serotonin had no significant effect on this enzyme. None of the gastrointestinal hormones had any significant effect on FDP aldolase activity.

Of the six GI hormones, glucagon, serotonin and secretin significantly altered hepatic cAMP levels. Glucagon and serotonin significantly increased cAMP levels 337% and 44%, respectively, while secretin significantly decreased cAMP levels 27%. The only GI hormone producing any significant effect on hepatic cGMP levels was serotonin, which produced a 20% increase above control levels.

These data demonstrate that GI hormones produce acute effects on plasma glucose levels and hepatic gluconeogenic and glycolytic enzyme activities and hepatic cyclic nucleotides. It appears that hepatic PK and PC are two regulatory enzymes of hepatic glycolysis

and gluconeogenesis, respectively, which are extremely sensitive to acute regulation by GI hormones. In contrast, hepatic PEPCK and FDPase (other regulatory enzymes of gluconeogenesis) do not appear to be as sensitive. Many of the acute changes noted are not due to changes in hepatic cAMP or cGMP levels, since three of the GI hormones (CCK-PZ, gastrin and histamine) had no significant effect on either of these two cyclic nucleotides. In most cases, there does not appear to be a high degree of correlation between the blood glucose levels and the various biochemical parameters. It is still essential to determine the insulin and glucagon levels and to compare the ratios of these two hormones with the changes in plasma glucose levels. These data are pending.

CONCLUSIONS:

Intravenous GI hormones do produce acute changes in blood glucose levels as well as in hepatic glycolytic and gluconeogenic enzyme activities and hepatic cAMP levels. Only serotonin significantly altered hepatic cGMP levels. With the exception of glucagon, there does not appear to be a consistent correlation between changes in hepatic gluconeogenic and glycolytic enzyme activities and blood glucose levels.

RECOMMENDATIONS:

Because of the importance of the rapid regulation of blood glucose levels by intravenous GI hormones, it is imperative to determine precisely what effects these hormones have on biochemical pathways involved in glucose production and utilization. Efforts should be continued on elucidating the biochemical mechanisms by which these GI hormones exert these rapid effects.

Studies will be continued with the most highly purified sources of these respective GI hormones available (obtained from Dr. Mutt courtesy of the National Institutes of Health).

PUBLICATIONS:

1. Stifel, F. B., H. L. Greene, E. G. Lufkin, M. R. Wrensch, L. Hagler and R. H. Herman. Acute effects of oral and intravenous ethanol on rat hepatic enzyme activities. *Biochim. Biophys. Acta* 428: 633, 1976.
2. Lufkin, E. G., O. D. Taunton, F. B. Stifel, F. D. Hofeldt, M. R. Wrensch, L. Hagler and R. H. Herman. Effect of triiodo-tyrosine on human jejunal glycolytic enzymes. *Proc. Soc. Exptl. Biol. Med.* 150: 410, 1975.

3. Greene, H. L., F. B. Stifel, L. Hagler and R. H. Herman. Comparison of the adaptive changes in disaccharidase, glycolytic enzyme and fructosediphosphatase activities after intravenous and oral glucose in normal men. *Amer. J. Clin. Nutr.* 28: 1122, 1975.
4. Stifel, F. B., E. G. Lufkin, L. Hagler, H. L. Greene, O. D. Taunton, M. Wrensch, C. L. Miller and R. H. Herman. Improvement in jejunal enzyme adaptation in obese adult-onset diabetic patients following a thirty-day fast. *Amer. J. Clin. Nutr.* 29: 989, 1976.

STUDY NO. 2b.

Effects of Intravenous Amino Acids on Plasma Glucose and Hepatic Gluconeogenic and Glycolytic Enzyme Activities in the rat.

PROBLEM:

Insulin is an acute regulator of hepatic glycolysis and gluconeogenesis. Specifically, intravenous insulin rapidly activates hepatic pyruvate kinase (PK) and inactivates hepatic fructosediphosphatase (FDPase) activities through mechanisms which do not appear to involve cyclic AMP.

It is well known that many amino acids stimulate the release of pancreatic insulin. Arginine, lysine, and leucine are three amino acids which exert such an insulinogenic response. Valine does not exert this effect. Alanine is a known potentiator of pancreatic glucagon which, in turn, causes acute responses that are reciprocal to those seen with insulin.

In this study, we examined the effects of five intravenously administered amino acids (alanine, arginine, leucine, valine and tryptophan) on blood glucose levels and attempted to correlate these changes (if any) with acute changes in hepatic glycolytic (PK and FDP aldolase) and gluconeogenic (FDPase, pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK)) enzyme activities. Plasma samples were also collected for determination of insulin and glucagon levels. Effective and efficient muscular performance is the hallmark of the soldier especially in basic training and combat. Muscular function depends, in part, on endogenously supplied fuels, viz., glucose and free fatty acids. The mobilization of such fuels during exercise depends on the recycling of lactic acid produced by exercising muscle through the gluconeogenic pathways of the liver to produce glucose, 2) the lipolytic enzymes of adipose tissue to produce free fatty acids, 3) glycolysis which produces glucose from hepatic glycogen, 4) the hormonal systems that regulate gluconeogenesis, lipolysis and glycogenolysis. Any defect in any of these component parts can lead to ineffective muscular function. See Study No. 3.

RESULTS AND DISCUSSION OF RESULTS:

Of the five amino acids, only alanine significantly increased plasma glucose levels (41%), while leucine and arginine significantly decreased plasma glucose levels 20% and 25%, respectively. Valine and tryptophan had no significant effect on plasma glucose levels.

The only significant changes in hepatic enzyme activities were as follows: arginine increased PC and PEPCK by 26% and 28%, respectively; alanine increased PC and FDPase by 17% and 49%, respectively, and decreased PK by 30%; and leucine decreased FDPase by 32%. The changes in hepatic FDPase activity appeared to correlate best with the changes in blood glucose levels. In four of the five instances, FDPase changes correlated well with the direction and magnitude of the blood glucose changes.

CONCLUSIONS:

Intravenously administered alanine, arginine and leucine produce acute changes in both plasma glucose levels and hepatic enzyme activities while valine and tryptophan do not. The changes in blood glucose levels appeared to correlate best with changes in hepatic FDPase (a gluconeogenic enzyme) activities.

RECOMMENDATIONS:

The evidence from this study suggests that the nature of the dietary protein source and its characteristic amino acid composition may be extremely important in terms of acute blood sugar regulation. It is important to determine the acute effects of other amino acids and their various combinations on these same parameters.

PUBLICATIONS: None.

STUDY NO. 3

Studies on the Regulation and Production of Fuel

PROBLEM:

The production and utilization of fuel for body metabolism during resting and exercise states is a complex system of metabolic processes involving the turnover and interaction of numerous substrates, hormones, and enzymes. The metabolic processes respond to exercise, resting, fed and fasting conditions. Dietary intake, which provides substrate for fuel (glucose, amino and fatty acids), affects the hormonal and the enzymatic adaptations that are necessary for the proper utilization of the substrates.

Failure of the appropriate hormonal and enzymatic adaptive changes during exercise, fasting or the fed state results in conditions

that lead to decreased performance because of failure of the brain and muscle to obtain necessary fuel. A number of patients have clinically evident disorders leading to hypoglycemia soon after consuming glucose. It is possible that a larger population of apparently normal soldiers could have sub-clinical disorders of a similar nature that might become evident only during the stress of exercise (e.g., during basic training or combat). A survey of 2,000 military recruits revealed that 23% had lower than normal serum glucose levels (below 60 mg/dl) 2 hours following ingestion of a glucose load. In order to determine whether or not abnormalities in the regulation of blood glucose levels as a consequence of food intake and/or exercise occurs, it is necessary to investigate the hormonal and enzymatic adaption in normal volunteers and selected patients under conditions of controlled diet and exercise. Previous studies have shown that folic acid is able to increase the activities of several enzymes which are critical in the regulation of blood glucose levels. It is possible that certain patients who have abnormalities of blood glucose regulation after eating and/or exercise may benefit from oral folate therapy. Military personnel in training and combat operations must eat the food provided and cannot select specific diets. Thus, it becomes essential to identify individuals who are intolerant to certain foods and exercise because of the resulting disturbance in blood glucose regulation.

RESULTS AND DISCUSSION OF THE RESULTS:

Three patients with reactive hypoglycemia and one with a suspected disorder of folate metabolizing enzymes have been studied. The patients with reactive hypoglycemia consumed liquid formula diets consisting of 50% carbohydrate, 20% protein and 30% fat. Jejunal and liver biopsies were obtained to measure glycolytic and gluconeogenic enzyme activities. Oral glucose tolerance, intravenous glucose tolerance and glycerol tolerance tests were performed. Following 1 week of therapy with 15 mg folate/day the biopsies and tolerance tests were repeated. In two patients the reactive hypoglycemia disappeared with the formula diet. In one patient, ad lib hospital and carbohydrate-free diets were associated with exacerbation of the reactive hypoglycemia after glucose ingestion. This patient had hyperinsulinism after oral glucose but not after intravenous glucose. No abnormalities were found after glycerol or fructose loading in any of the patients. None of the patients had hepatic fructose-1,6-diphosphatase deficiency which can cause varying degrees and types of hypoglycemia. In one patient mild post-exercise hypoglycemia occurred. Studies were done in one patient with low serum folate, macrocytosis of red blood cells, poor response to folate therapy and no apparent dietary cause for folate deficiency. No abnormality in utilization of glucose was found. The cause of the condition has not been determined.

CONCLUSIONS:

It is clear that hypoglycemia may occur as a consequence of glucose ingestion following a low carbohydrate diet. This does not ordinarily occur in normal individuals. In the one patient with hyperinsulinism and hypoglycemia after oral carbohydrate ingestion but no abnormal insulin response and no hypoglycemia after intravenous glucose it is possible that some gastrointestinal factor was released from the GI tract in response to oral carbohydrate which in turn increased the secretion of insulin. Exercise can produce mild hypoglycemia by a different mechanism. Increased utilization of glucose by muscle in excess of glucose production by the liver may be responsible for exercise-induced hypoglycemia but there is suggestive evidence that some factor other than insulin may be implicated.

RECOMMENDATIONS:

Further studies are needed to determine 1) the nature of the gastrointestinal factor(s) which might be responsible for insulin secretion, 2) the nature of gluconeogenetic failure in providing sufficient glucose after eating and during exercise (such as occurs with FDPase deficiency), 3) the mechanism whereby exercise lowers blood glucose levels, 4) the mechanism whereby folic acid increases hepatic enzyme activities, and 5) the significance of low blood glucose in military recruits after eating and exercise.

PUBLICATIONS:

Hofeldt, F.D., E.G. Lufkin, S.F. Hull, J.W. Davis, S. Levin, and P.H. Forsham. Alimentary reactive hypoglycemia; effects of DBI and Dilantin on insulin secretion. Military Medicine 140: 841-845, 1975.

STUDY NO 5

Studies of a Patient with Hypertension

PROBLEM:

Essential hypertension is a chronic, frequently asymptomatic illness affecting 20-30 million Americans (approximately 10% of the general population). It results in significant morbidity and mortality in the form of strokes, myocardial infarctions, congestive heart failure, atherosclerosis and renal failure when untreated. The terminology "essential hypertension" refers to that group of patients in whom no definite hormonal or anatomic etiology is demonstrable. In a minority of patients with hypertension, an over-production of one of the hormones affecting sodium and water balance (and hence the blood pressure) can be demonstrated. Some of the hormones that may cause hypertension can also affect the glucoregulatory mechanisms that have been the subject of considerable interest in our laboratory

(see Work Unit No. 156, Study No. 3). A patient with an unusual case of severe hypertension that was unresponsive to conventional forms of therapy was referred to the Metabolic Ward, LAIR, by physicians from the Department of Medicine, Letterman Army Medical Center, LAMC, for evaluation of a possible hormonal abnormality causing hypertension.

RESULTS AND DISCUSSION OF THE RESULTS:

A 29 year old female Army nurse had progressively severe hypertension over a one year period. She had sustained hypertension ranging from 160-200 systolic/100-160 diastolic while on maximal doses of conventional antihypertensive medications. Several times weekly the blood pressure rose to the upper limits mentioned above and she experienced a flushing sensation and headaches. During those episodes Diazoxide, 300 mg intravenously, lowered the blood pressure transiently. Twenty-four hour urine for vanilylmandelic acid, metanephrine and epinephrine were within the normal ranges. During Regitine^(R) infusion the blood pressure dropped significantly (30 mm Hg systolic and 40 mm Hg diastolic) but there was no drop in serum glucose which indicated a false positive response to Regitine^(R). No evidence for an adrenogenital syndrome, Cushing's disease and hyperaldosteronism was found. Abdominal ultrasound, renal arteriograms and measurements of renin, aldosterone, plasma epinephrine, and aldosterone effect by a binding assay were normal. The patient's cortisol level suppressed adequately during treatment with dexamethasone. High (4 grams) and low (less than 1 gm) sodium diets did not affect the blood pressure. The patient had oligomenorrhea. She had normal testosterone and estradiol levels but elevated levels of luteinizing hormone. Urinary pregnanetriol was 1.1 mg/24 hrs. (normal 3.5 or less).

CONCLUSION:

The cause of this patient's hypertension could not be determined. The relationship of possible hormonal or enzymatic abnormalities to the hypertension was likewise not determined although further tests are in progress.

RECOMMENDATIONS:

The Metabolic Ward, LAIR, is available to assist the physicians at LAMC in the study of patients who have disease that is difficult to diagnose and/or to treat. In accordance with the host-tenant agreement between LAIR and LAMC, we provide facilities for the study of such patients. This is an example of the type of sophisticated study that can be done to extend the capabilities of LAMC to provide the best medical treatment that is available. We should continue to support the efforts of the LAMC physicians in the medical care of military personnel particularly in those areas that we are especially equipped to handle. This gives us the opportunity to study medical problems that are related to our on-going research efforts.

PUBLICATIONS: None.

STUDY NO. 6

The Study of a Patient with
Idiopathic Bone Disease Resembling
Stress Fractures

PROBLEM:

Two types of fractures occur with high incidence in the military: (1) fractures resulting from a sudden violent force and (2) the so-called march or stress fracture. The cause of the first type is readily understood; the second type has a less obvious etiology. During World War II, 1,881 stress fractures were diagnosed at Camp Wheeler, Georgia, and Ft Wolters, Texas. Seven hundred stress fractures were reported at Ft Ord, California, between 1962-1965. During 1966, 250 cases of stress fractures were seen at Ft Benning, Georgia. In one study of 33 cases at Ft Dix, New Jersey, most patients could not be returned to duty in less than 6 months and 30% were never able to return to duty. The magnitude of the problem, the tremendous loss of productivity and the expense of such injuries are of major military importance.

Stress fractures are attributed to failure of the bone to withstand prolonged and unaccustomed physical effort. It is unknown why some recruits are more susceptible to such fractures. It is possible that some recruits have a subtle form of metabolic bone disease that predisposes to such injury. Vitamin D plays a key role in bone remodeling and thus in the adaptation of the bone to stress. Recent advances in the understanding of vitamin D metabolism have led us to undertake studies to examine the role of vitamin D and its more active metabolites in bone formation, resorption, healing and adaptation to stress. The referral of a patient with unexplained fractures presumably due to stress provided an opportunity to evaluate some of these relationships.

RESULTS AND DISCUSSION OF THE RESULTS:

A 45 year old black female with obesity, hypertension and 3 documented fractures related to minimal or no trauma was referred from the Orthopedic Service, LAMC, to the Metabolic Ward, Department of Medicine, LAIR, for evaluation. Calcium balance studies were done while the patient was on a liquid formula, low calcium diet (350 mg/d) and a high calcium diet (1.0 gram/d) (phosphorous content, 1 gram/d during both periods). The results of the calcium balance studies are pending. At the close of each period duodenal tissue was obtained by peroral biopsy for measurement of calcium uptake (CaU), phosphorous uptake (PU), and tissue alkaline phosphatase (alk Pase). On the 350 mg calcium diet CaU, PU and alk Pase were 5.74 nmoles/mg, 13.72 nmoles/mg, and 34.37 nmoles/min/ μ g protein, respectively. On the 1 g calcium diet CaU, PU and alk Pase were 2.76 nmoles, 14.54 nmoles/mg, and 30.26 nmoles/min/ μ g

protein, respectively. During the evaluation of the patient's problem, elevated levels of cortisol were found. A left adrenal adenoma was discovered and removed surgically. Clearly, this was a patient with Cushing's syndrome manifested primarily by bone disease which mimicked stress fractures. Her hypertension which had been thought to be essential hypertension clearly was part of the Cushing's syndrome.

CONCLUSIONS:

Study of this patient gave us the opportunity to understand better the interrelationships between vitamin D, CaU, PU, catabolic effects of adrenocortical steroids and stress fractures.

This patient's problem emphasizes the importance of adrenocortical excess in the development of bone disease and the lack of healing that results when excess cortisol is present. This adds an additional dimension to our studies of the involvement of vitamin D and its derivatives in the pathogenesis and possible treatment of stress fractures.

RECOMMENDATIONS:

Additional studies are required to define the role of vitamin D and its metabolites and other hormone, such as cortisol, on bone remodeling and the response to stress. Studies in patients with stress fractures, in normal volunteers, and in patients with other forms of bone disease should be carried out in order to define these relationships. A protocol (old work unit 059, new work unit 070) has been submitted to study the role of vitamin D in the etiology and treatment of fractures. See also Work Unit No. 059 for the supporting animal studies.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISB'N INSTR'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
75 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62760A	3A762760A822		01		157	
b. CONTINUING ^a							
c. CONTINUING ^a	CARDS 114 (F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Studies on Blistering Produced by Mechanical, Thermal, and Chemical Agents (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
0035-0 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
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17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
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Not Applicable				77		0	
e. KIND OF AWARD:		f. CUM. AMT.				0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Allen, A.M., LTC, MC			
				NAME: Akers, W.A., COL, MC			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Prevention; (U) Feet; (U) Skin; (U) Blistering machine; (U) Human volunteers; (U) Friction; (U) Blisters; (U) Training camps							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To study the epidermis, its chemical nature and physical properties when subjected to frictional injury in order to find ways to raise the threshold of susceptibility to skin frictional injury and reduce the disability and sequelae produced from blisters incurred by soldiers on long marches.							
24. (U) Experimental friction blisters by linear and twist rubbing will be used as a model of studying the prevention and treatment of friction blisters. Field studies are now in progress which are designed to answer the following questions: What is the prevalence of friction blisters at various times during the basic training cycle? What percent of blisters become infected? What percent of soldiers with blisters report to the dispensary? What percent of recruits with infected blisters are hospitalized? Where is the precise location of blisters on the foot? Is there a particular location where blisters are likely to become infected? Is there a particular location where a blister is not likely to become infected? Is there a critical size relationship to infection? Is there a way to predict which soldier will develop blisters? Do weather conditions affect the incidence of blisters?							
25. (U) 75 07 - 76 09 Liason visits were made to Fort Ord, Ft. Leonard Wood, Lackland AFB, and the Marine and Navy Recruit Training Center, at San Diego to develop studies in determining the natural history, epidemiology, and location of friction blisters in recruits. Later glutaraldehyde and other friction reducing modifiers will be evaluated. This work unit will terminate but the studies will continue as part of the new work unit 002, beginning 1 Oct 1976.							

^a Available to contractors upon originator's approval.

312

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	157	Studies on Blistering Produced by Mechanical, Thermal, and Chemical Agents
STUDY NO.	1	Epidemiology of Friction Blisters in Soldiers

Liaison visits were made to Fort Ord, Fort Leonard Wood, Lackland Air Force Base, and the Marine and Navy Recruit Training Center at San Diego to design studies on the natural history, epidemiology, and location of friction blisters in recruits. Later glutaraldehyde and other friction-reducing modifiers will be evaluated. This work unit will terminate but the studies will continue as part of the new Work Unit 002, beginning 1 Oct 1976.

BODY OF REPORT

WORK UNIT NO. 157

Studies on Blistering Produced by
Mechanical, Thermal, and Chemical
Agents

STUDY NO. 1

Epidemiology of Friction Blisters in
Soldiers

PROBLEM:

Friction blisters cause high rates of disability in the military, especially among recruits where 10% are placed on quarters and 1% are hospitalized. Seasoned troops suddenly relocated into hot climates may develop blisters which can compromise a commander's ability to accomplish his mission. Recent visits were made to Fort Ord, Fort Leonard Wood, Lackland Air Force Base, and the Marine and Navy Recruit Training Center, San Diego. Friction blisters and their sequelae (cellulitis and infection) were confirmed to be a major medical problem at all these bases and a factor in loss of training time. In addition to the morbidity of the soldier, there is considerable economic cost to the services for hospitalization and recycling for training.

RESULTS AND DISCUSSION OF THE RESULTS:

The primary objective of this study is to determine prospectively the natural history and epidemiology of friction blisters. The study is designed to answer the following questions: (1) What is the incidence of friction blisters after the first long hike among basic trainees? (2) What percent of blisters become infected? (3) What percent of men with infected blisters are hospitalized? (4) Where is the precise location of blisters on the feet? (5) Is there a particular location where blisters are likely to become infected? (6) Is there a critical size relationship to infection? (7) Is there a way to predict which soldier will develop blisters?

CONCLUSIONS:

From the results of this investigation, the therapeutic and prophylactic efficacy of glutaraldehyde and other topical agents can easily be determined by an identical study comparing them with untreated controls.

RECOMMENDATIONS:

The field survey will begin 7 Sept 1976 and the prophylaxis study will begin next spring.

PUBLICATIONS:

Maibach, H.I., and S.D. Prystowsky: Glutaraldehyde (pentanedial) allergic contact dermatitis: usage test on sole and antecubital fossa - regional variations in response. Arch Dermatol (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISB'N INSTR'N	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO. / CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A811				011	
b. CONTRIBUTING	62760A	3A762760A822		01		158	
c. COLLABORATING	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code)* (U) The Metabolic Responses of the Gastrointestinal Tract to Dietary Substances, Drugs and Hormones in Health and Disease. (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
74 12		CONT		DA		C. In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER* Not Applicable				76		4	
c. TYPE:				77		1	
e. KIND OF AWARD:				CURRENT		4	
f. CUM. AMT.				77		98	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME* Herman, R. H., COL, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., COL, MC			
				NAME: POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Gastrointestinal Disease, Jejunal Enzymes, Adaptive Responses of Jejunal Enzymes, Diarrheal Syndromes; (U) Human Volunteers; (U) Laboratory Animals.							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23.(U) Acute and chronic gastrointestinal (G.I.) diseases affect military personnel causing long-term morbidity, loss of duty time and high costs of continuing in- and out-patient care. In 1966 in Vietnam diarrheal diseases accounted for 12% of all hospital admissions. In World War II functional dyspepsia and chronic idiopathic diarrhea accounted for 15-20% of admissions to war zone hospitals and 62% of these patients were returned to the U.S. for treatment. Acute diarrhea is disabling and difficult to prevent since the etiology is often unknown (87% of 1,954 patients in RVN, May 1965). Chloroquin, a potent anti-malarial agent, often causes acute diarrhea leading to non-compliance and hence ineffective malarial prophylaxis. G.I. injury from combat wounds precludes enteral feeding and results in atrophy of the small intestine, pancreas and liver. Atrophy may be due to absence of gastrin which results from lack of enteral feeding or traumatic loss of gastrin secreting tissue. Gastrin replacement might reverse G.I. atrophy and enhance rehabilitation. 24.(U) We will study: 1) The response of G.I. enzymes to diet, folic acid and chloroquin in patients with chronic idiopathic diarrhea; 2) The effect of enterotoxins and chloroquin on animal jejunal mucosal enzymes <u>in vitro</u> ; 3) The effect of gastrin administration on G.I. glycolytic enzymes in gastrin deficient patients; and 4) The ability of gastrin to enhance healing of G.I. injury in suitable patients. 25(U) 75 06-76 09 Jejunal mucosa from the pig and guinea pig <u>in vitro</u> incorporate ¹⁴ C-leucine into protein, ¹⁴ C-thymidine into DNA and ¹⁴ C-uridine into RNA. ¹⁴ C-leucine incorporation into protein is inhibited by actinomycin D and cycloheximide. Animal jejunal mucosa can be used to study the action of nutrients, enterotoxins and hormones on the metabolic processes involved in acute and chronic diarrhea. We will extrapolate these results to study human G.I. mucosa <u>in vitro</u> in similar fashion.							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	158	The Metabolic Responses of the Gastrointestinal Tract to Dietary Substances, Drugs and Hormones in Health and Disease.

The following investigations have been conducted under this work unit:

STUDY NO. 1. In Vitro Study of Control of Small Intestinal Mucosal Enzymes.

Using an in vitro gut incubation system, rabbit, guinea pig, swine, monkey and chicken intestine have been cultured for periods up to 24 hours. We have demonstrated incorporation of ^{14}C -leucine, ^{14}C -thymidine and ^{14}C -uridine into perchloric acid (PCA) insoluble material, presumably protein, DNA, and RNA, respectively. Current investigation involves the use of metabolic inhibitors of these processes.

BODY OF REPORT

WORK UNIT NO. 158

The Metabolic Response of the
Gastrointestinal Tract to Dietary
Substances, Drugs and Hormones in
Health and Disease

STUDY NO. 1

In Vitro Study of Control of
Small Intestinal Mucosal Enzymes

PROBLEM:

In order to devise an in vitro system for studying gastrointestinal mucosal metabolic activities, we have been testing animal small intestinal mucosa. If we can devise an in vitro system using animal small intestinal mucosa which is metabolically active, we may be able to employ a similar system in the study of human small intestinal mucosa obtained by peroral biopsy from normal volunteers and patients with gastrointestinal disease. At present, it is necessary to hospitalize patients on our metabolic ward for several weeks and to obtain small intestinal tissue by peroral biopsy after carefully controlled dietary periods in order to test the effect of diet on small intestinal enzymes. If we can obtain similar data by using small intestinal tissue incubated in vitro, hopefully, we will be able to shorten the hospital stay, decrease the expenditure of money, and enlarge the scope of testing of the tissue that would otherwise not be possible in vivo. Since acute and chronic diarrhea are important military problems, it is essential to be able to study the effect of various medications (e.g., chloroquin), bacterial agents (e.g., enterotoxins), hormones (e.g., gastrin), and dietary substances on the mechanisms in the small intestine that have been implicated in acute and chronic diarrhea. As a first step, we are developing a means of incubating animal small intestinal mucosa in vitro so that such tissue remains viable and metabolically active over a sufficient length of time to allow the appropriate studies to be accomplished. The present studies are designed to test the viability of small intestinal mucosa from various animal species. A good test of viability is the ability of such tissue to incorporate ^{14}C -leucine into protein, ^{14}C -thymidine into deoxyribonucleic acid (DNA) and ^{14}C -uridine into ribonucleic acid (RNA). The effect of inhibitors with known mechanisms of action will show that such incorporation is a function of normal metabolic processes. We then can test the effect of various nutrients (amino acids, sugars, fats, folic acid), hormones (e.g., gastrin), medications (e.g., chloroquin) and enterotoxins on glycolytic and gluconeogenic enzymes and other appropriate enzymes of such tissue.

RESULTS AND DISCUSSION OF THE RESULTS:

We have found that pig small intestinal mucosa incorporates ^{14}C -leucine, ^{14}C -thymidine and ^{14}C -uridine into perchloric acid insoluble material,

i.e. into protein, DNA, and RNA, respectively. Actinomycin D and cycloheximide inhibit ^{14}C -leucine incorporation into protein. Maximum incorporation occurred when the incubation media was supplemented with glutamine, adenine, guanine, and cytosine. Similar results have been obtained in the guinea pig. Although rabbit small intestinal mucosa incorporated ^{14}C -leucine into protein, such incorporation was relatively slow and ^{14}C -thymidine incorporation into DNA was negligible. The incorporation of labelled substances into protein and nucleic acids was continuous and linear for periods up to 24 hr in the pig and guinea pig mucosa which indicates their viability.

CONCLUSIONS:

The incubation of small intestinal mucosa in vitro has been shown to maintain pig and guinea pig intestine for periods up to 24 hrs. Continuous, linear incorporation of labelled thymidine, leucine and uridine into DNA, protein, and RNA have been demonstrated.

RECOMMENDATIONS:

These studies should be continued. Metabolic inhibitors of these processes should be used to demonstrate that normal metabolic processes are occurring. The effect of nutrients, hormones, medications, and enterotoxins on these processes and various intestinal enzymes should be tested. Application of these techniques to human small intestinal mucosa obtained by peroral biopsy would appear to be possible.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISB'N INSTR ^a	9b. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
76 04 02	H. Termination	U	U	NA	NL		
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62760A	3A762760A822		01		160	
b. XXXXXXXXXX							
c. XXXXXXXXXX	CARDS 114 (F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Clinical Evaluation of Alpha-Cyanoacrylates and Treatment of Friction Blisters (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 05		76 09		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER: Not Applicable				FISCAL YEAR		76	
c. TYPE:				CURRENCY		0.0	
d. KIND OF AWARD:						1	
e. AMOUNT:				77		0	
f. CUM. AMT.						0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				Department of Dermatology Research			
				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Akers, W.A., COL, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5455			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Isoamyl-cyanoacrylate; (U) Blister therapy; (U) Topical; (U) Laboratory animals							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number, precede text of each with Security Classification Code)							
<p>23. (U) To develop a method of therapy for friction blisters of the feet which can be used by the soldier in the field to reduce ineffectiveness, pain, infection, and to permit healing.</p> <p>24. (U) Clinical evaluation of the cutaneous reactivity and sensory response to the topical application of cyanoacrylate homologues on experimental friction blister bases. To conduct clinical trials in soldiers to prove safety and efficacy of isoamyl cyanoacrylate for treating torn or raw friction blisters as compared to current standard therapy.</p> <p>25. (U) 75 07 76 09. Prior to conducting a large scale clinical trial among soldiers for effectiveness, the Food and Drug Administration will require lifetime studies in mice and rats of isoamyl cyanoacrylate implanted subcutaneously. There is concern about the Oppenheimer or solid-state carcinogenic phenomenon associated in these animals with glass, various metals, and various plastics. Isoamyl cyanoacrylate is not entrapped in tissue when used to treat blisters but could become imbedded in treating cuts, scratches, or abrasions. This work unit will terminate, due to lack of funds to complete the required studies.</p>							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT.	160	Clinical Evaluation of Alpha-Cyanoacrylates and Treatment of Friction Blisters

A consultant visited us in August 1975 and offered advice on developing a protocol for this work unit. Before field trials can begin, solid state carcinogenesis studies in mice (18 months) and rats (24 months) must be conducted in accordance with Food and Drug Administration regulations. Sufficient polymer to conduct the carcinogenesis studies was received from the U.S. Army Medical Bioengineering Research and Development Laboratory. Funds are not available to do the animal studies. No clinical evaluations were performed during the past year. Evaluation of cyanoacrylate should be done because if it proved safe, it would offer the soldier a liquid "bandaid" that can be carried into the field for use in the treatment of friction blisters, abrasions, cuts and scratches.

This work unit will terminate, but the studies will be combined into a new Work Unit 002, "Prevention of Common Skin Diseases Caused by Environmental Assaults on the Soldiers Skin", beginning 1 Oct 1976.

BODY OF REPORT

WORK UNIT NO. 160

Clinical Evaluation of Alpha-Cyanoacrylates and Treatment of Friction Blisters

PROBLEM:

We are seeking a field treatment for raw friction blister bases, and minor skin injuries on the feet of soldiers. Present treatment consists of topical antibiotic ointments and bandages that must be applied several times a day but these are not carried into the field. Isoamyl cyanoacrylate, a liquid adhesive has performed well in laboratory test in volunteers and on soldiers in two small field trials. It lessens pain and infection, permits walking, and one application adheres for six days. To conduct a large field trial and to permit the isoamyl cyanoacrylate to be used on scratches, abrasions, minor cuts, and skin donor sites where the material could be entrapped in the dermis, the Food and Drug Administration will require solid state carcinogenesis studies in mice (18 months) and rats (24 months). Whenever a disc of solid material is implanted subcutaneously or intraperitoneally in mice or rats for at least 6 months and the foreign material excites the formation of a fibrous capsule, a sarcoma can develop from the capsular wall and metastasize. All solid plastics, glass, metals, and cellophane can produce the sarcomas.

RESULTS AND DISCUSSION OF THE RESULTS:

In order to do the carcinogenesis studies in-house, it would cost about \$150,000 which has not been available. From 1200 to 1400 animals will be required. We have all the facilities available to do the studies.

RECOMMENDATIONS:

Work Unit is being terminated. If money becomes available to complete the required tests, the studies will be conducted under a new Work Unit.

The studies should be done because the cyanoacrylate offers the soldier a liquid "bandaid" that can easily be carried into the field and used.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6912	76 09 30	DD-DR&E(AR)636	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	H.Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62760A	3A672760A822		01		162	
B. XXXXXXXXXX							
C. XXXXXXXXXX	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code) ^a (U) Studies on the Effects of Heat and Humidity upon the Human Skin with Particular Emphasis on Prickly Heat & Consequent Disabling Dermatoses							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 08		76 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
D. NUMBER:				76		0	
E. TYPE: Not Applicable				FISCAL		0	
F. CUM. AMT.				77		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Department of Dermatology Research			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Prystowsky, S.D., MAJ, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Grekin, D.A., MAJ, MC			
				NAME: Akers, W.A., COL, MC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Heat Retention; (U) Stratum Corneum; (U) Heat Fatigue; (U) Human volunteer; (U) Miliaria; (U) Prickly heat; (U) Rash; (U) Sweating; (U) Skin							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To find the casual mechanism, means of prevention, treatment, and the interrelationship of prickly heat rash (miliaria) to disturbances in sweating (hypohidrosis) due to heat retention and heat fatigue in soldiers.							
24. (U) Volunteers prone or resistant to experimental prickly heat and subsequent decrements in sweating are characterized regarding skin bacteria, skin surface fats, skin pH, and hydration dynamics of the horny layer. Various prophylactic and therapeutic modalities will be tested and evaluated.							
25. (U) 75 07 - 76 09 The literature on miliaria has been reviewed. A study protocol is being developed to define the role of cutaneous bacteria in producing miliaria. The development of the rapid and quantitative replicate plating bacteriological method under Work Unit 010 has made this study feasible. This work unit will terminate but will continue as part of the new Work Unit 002 beginning 1 Oct 1976.							

* Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	162	Studies on the Effects of Heat and Humidity Upon the Human Skin with Particular Emphasis on Prickly Heat and Consequent Disabling Dermatoses

A study protocol is being developed to investigate the inter-relationship of miliaria to hypohidrosis and heat fatigue in soldiers. The role of cutaneous bacteria in the pathogenesis of miliaria will be further defined.

BODY OF REPORT

WORK UNIT NO. 162

Studies on the Effects of Heat and Humidity Upon the Human Skin with Particular Emphasis on Prickly Heat and Consequent Disabling Dermatoses

The literature has been reviewed and a study protocol is being written to resume the investigations, particularly regarding the role of occlusion and the skin microflora in causing miliaria (prickly heat rash). These studies will mesh with other studies on water immersion and cutaneous microflora ecology. Since we know that topical chloramphenicol, neomycin, and anhydrous lanolin preserve the patency of the sweat duct orifice against experimentally induced miliaria, other antibiotics and greasy substances will be investigated.

This work unit will terminate But the studies will be combined into a new Work Unit 002 "Prevention of Common Skin Diseases Caused by Environmental Assaults on the Soldiers Skin" beginning 1 Oct 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OB 6912	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISB'TN INSTR'TN	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A810				004	
b. XXXXXXXXXX	62760A	3A672760A822		01		163	
c. XXXXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code*) (U) Role of Skin Lipids in Prevention and Control of Infectious Diseases in Military Personnel (01)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 07		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
EXPIRATION:				76		2.0	
b. NUMBER*				FISCAL		.5	
c. TYPE				77		86	
Not Applicable				CURRENT			
d. AMOUNT:				77		3.0	
e. CUM. AMT.							
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Cutaneous Protection Division			
				Department of Dermatology Research			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME* Schmid, P., Ph.D, DAC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Fungal Infections; (U) Water Immersion; (U) Biochemistry; (U) Lipids; (U) Human volunteers; (U) Cholesterol							
23. (U) To study the composition of skin lipids and other cutaneous substances and to study their interaction on the skin surface of soldiers. To study these parameters and others in relation to militarily important skin diseases, such as fungal infection, or in relation to military casualties due to hot and wet, hot and dry climates, or military clothing. To test or develop more effective therapeutic agents for the treatment of skin diseases.							
24. (U) The project will be developed in two areas: (1) develop or improve techniques to determine the composition and/or quantity of skin lipids and other skin substances, (2) apply the evolved techniques to the study of bacterial and/or fungal infections and to other causes of casualties in military populations.							
25. (U) 76 04 - 76 09 Studies on characterizing and quantitating potential inhibitors or promoters of fungal infection (<i>Trichophyton mentagrophytes</i>) in skin lipid extracts of feet continued. It was demonstrated that volunteers who were chronically infected with <i>Trichophyton</i> had a cholesterol content per area which was not statistically significantly different from that of volunteers without fungal infection. A new wet-ashing procedure for oxidation of fungal lipids was developed which is devoid of the explosion hazard of perchloric acid. Additional computer programs were developed to allow efficient calculation, statistical evaluation and updating of the data bank on skin lipids. It was shown unequivocally that phospholipids are not a significant component in skin lipids.							

* Available to contractors upon originator's approval

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	00	Internal Medicine
WORK UNIT NO.	163	Role of Skin Lipids in Prevention and Control of Infectious Disease in Military Personnel

The following investigations have been conducted under this work unit:

- STUDY NO. 11 Cholesterol Content of Skin Lipids in Relation to Fungal Infection
- STUDY NO. 12 Wet Combustion of Lipids without the Use of Perchloric Acid
- STUDY NO. 13 Determination of Inorganic Phosphorus with an Ion-selective Electrode
- STUDY NO. 14 User-oriented Statistical Computer Programs
- STUDY NO. 15 Preliminary Data on Phospholipid Content in Relation to Fungal Infection

New advances in developing countermeasures for militarily important dermatological problems depend heavily on fundamental research into biological and biochemical processes.

Studies No. 11 and 15 Evidence from these studies indicates that the amount of cholesterol is high whereas, the amount of phospholipid is extremely low. This suggests that a hitherto unknown mechanism exists which recycles phospholipids of epidermal cells. The implication of these findings is not known at this time.

Study No. 12 A method of wet-ashing biological tissue was developed which eliminates potential explosions and sample loss encountered with the use of perchloric acid.

Study No. 13 The use of a new selective electrode for the measurement of phosphate was unsuccessful.

Study No. 14 User-oriented statistical computer programs were successfully used in connection with our study on skin lipids in relation to fungal disease.

BODY OF REPORT

WORK UNIT NO. 163

Role of Skin Lipids in Prevention
and Control of Infectious Disease
in Military Personnel

STUDY NO. 11

Cholesterol Content of Skin Lipids
in Relation to Fungal Infection

PROBLEM:

The means by which fungal infections are acquired and maintained in military populations are not entirely known. It is still not clear why some individuals are apparently resistant to fungal infection while others are chronically infected by fungi. Inhibitors that prevent the colonization of the sole and toes of the foot by fungi and bacteria have often been postulated but they have not been completely identified. In contrast, we hypothesize in this study that alteration in membrane structure and function by such agents as cholesterol may change resistance to fungal infection. The present study provides a quantitative evaluation of this hypothesis.

RESULTS AND DISCUSSION OF THE RESULTS:

Cholesterol was determined in skin lipid extracts based on our modification of the classical method of Lieberman and Burchard. In order to increase accuracy of results, the time dependence of the absorbance of the cholesterol chromophore system was taken into account in our calculations. Special computer programs were written to calculate data efficiently and to update the data bank on fungal disease of feet. Percent cholesterol in our lipid extracts is of the same order of magnitude as the percent reported for skin extracts from other parts of the body. The cholesterol value per area for the toe portion is lower than the value for the sole portion of the foot which indicates that sebaceous gland lipids may make an important contribution to the toe portion of the foot. This difference was found in all extracts of the three groups of volunteers, i. e. those who never had an infection (V), those who had no active lesion (E), and those who had a chronic infection (C). Our data indicate that there is no statistically significant difference in cholesterol content per area between groups V and E, Groups V and C, and groups E and C. This is true for extracts from the toe portion as well as the sole portion

CONCLUSIONS:

Volunteers who are chronically infected with Trichophyton mentagrophytes have a cholesterol content per area which is not statistically

significantly different from that of volunteers without any infection. This suggests that membrane structure and function of cells at the skin surface is similar for infected and uninfected skin. It is important to note that our data provide evidence that sebaceous gland lipids contribute a significant amount of skin lipids to the toe portion of the foot. This lipid may contain unknown inhibitors.

RECOMMENDATIONS:

Investigation of the causes of fungal disease of the skin of military personnel should continue and previously collected lipid extracts from volunteers with and without fungal infection should be used in the studies.

PUBLICATIONS:

None.

STUDY NO. 12

Wet Combustion of Biological
without the Use of Perchloric Acid

PROBLEM:

Concentrated perchloric acid alone or in combination with other oxidizing agents, such as nitric acid, are frequently used for wet combustion of biological material. However, it is well known that perchloric acid is dangerous because of the explosion hazard especially in combination with lipids. Because no stainless steel hood, which is mandatory, was available to our laboratory and because of the explosion hazard, a safer procedure for the microanalytic mineralization of phosphorus in lipid extracts was needed.

RESULTS AND DISCUSSION OF THE RESULTS:

Lipids dissolved in an organic solvent were added to conical centrifuge glasses and the organic solvent was evaporated in a tube heater which was subsequently used for wet-ashing. Combustion, performed with nitric acid and various mixtures of sulfuric and nitric acid, were not satisfactory for various reasons. However, the use of sulfuric acid in combination with hydrogen peroxide resulted in reproducible, complete oxidation of samples. Subsequent phosphate determinations by a conventional method were reproducible provided polysulfates were hydrolyzed prior to the determination.

CONCLUSIONS:

Our technique developed for the wet-combustion of organic material

is a valuable new tool because it is safe, simple, and requires no special equipment.

RECOMMENDATIONS:

For safety reasons, wet-ashing with perchloric acid should be discontinued and the new procedure used.

PUBLICATIONS:

None.

STUDY NO. 13

Determination of Inorganic Phosphorus
with an Ion-selective Electrode

PROBLEM:

Standard methods of analysis of biological phosphorus requires wet-ashing (see Study No. 12), followed by determination of inorganic phosphate by a method such as Fiske-Subba Row. These commonly used methods are time-consuming and involve a multitude of steps and equipment. Reduction of time to determine phosphorous would be cost-effective. Recently, an ion specific electrode for phosphate was made available commercially which promised a simple and efficient measurement of phosphate ions.

RESULTS AND DISCUSSION OF THE RESULTS:

Determinations on sodium salts of phosphoric acid proved extremely pH dependent. Selected organic buffers could be used to stabilize pH and make measurements more reproducible. However, in combination with acids (such as perchloric, sulfuric, nitric), ion selectivity for phosphate could not be demonstrated even when the pH was properly adjusted.

CONCLUSIONS:

Our results do not indicate that the commercially available ion-specific electrode can be used successfully to determine inorganic phosphate in solutions of wet-ashed organic material.

RECOMMENDATIONS:

These studies are potentially of great importance in biological chemistry and should be continued if and when newer solid state electrodes become available.

PUBLICATIONS:

None.

PROBLEM:

Complete and competent evaluation of laboratory or field generated results is an essential part of laboratory management. We have used and updated a sophisticated calculator system for a number of years. Simple programs were written mainly by laboratory technicians and investigators. Much duplication of efforts arose because of high turnover rate of military personnel and insufficient systematization. Furthermore, many experiments were not evaluated to the fullest extent because some laboratory personnel did not have the time or willingness to learn to operate the computing equipment. This demonstrated the need for a "turn-key" system which was easy to use by inexperienced personnel and which required minimum training.

RESULTS AND DISCUSSION OF THE RESULTS:

A set of computer programs was devised so that untrained personnel can make statistical analysis by the following computer generated instructions printed out on a data terminal and/or display. Instructions for use of programs are recorded on a single tape cassette.

CONCLUSIONS:

A user oriented statistical data system was designed and has been in operation for several months. The system produces more efficient and faster analysis of laboratory results. Untrained personnel can operate the system.

RECOMMENDATIONS:

A report titled "User Oriented Statistical Data System of the Dermatology Research Department" demonstrated that this system approach to data analysis should be implemented as a departmental Standing Operating Procedure.

PUBLICATIONS:

Schmid, P., J. Nonomura and K. McCumsey: User oriented Statistical data system of the Department of Dermatology Research. Published for Standing Operating Procedure.

STUDY NO. 15

Preliminary Data on Phospholipid
Content in Relation to Fungal
Infection

PROBLEM:

Fungal infections were occurring in epidemic proportions in military forces in Vietnam. However, the role of phospholipids in relation to dermatophyte infections is unknown. Furthermore, reports on phospholipids of the skin are scant and mostly misleading because of inadequate sampling techniques. Since phospholipids and cholesterol are vital constituents of membranes of most cells, quantitative study of phospholipids in lipid extracts of volunteers with no fungal infection or chronic fungal infection of their feet was therefore initiated.

RESULTS AND DISCUSSION OF THE RESULTS:

Lipid extracts from the skin of the foot contain extremely small quantities of phospholipids. It was unequivocally established that the low phospholipid content was not an artifact but that extraction of phospholipid was complete. Since appreciable quantities of cholesterol were found in the same extracts, and since cholesterol is a normal constituent of epidermal membranes but is found only in small amounts in sebaceous glands, the data suggest that organic phosphorus is apparently resorbed by some unknown mechanism. As a consequence and in the interest of human body economy, organic phosphorous compounds are not lost from the skin surface.

CONCLUSIONS:

Through techniques developed in this laboratory, it could be shown unequivocally that phospholipids are not a significant and normal part of skin lipids. This important finding suggests that a hitherto unknown resorption mechanism allows reutilization of phosphorous from fully differentiated epidermal cells.

RECOMMENDATIONS:

These studies on skin lipids are potentially of great importance. Since they suggest a hitherto unknown mechanism for resorption of phosphorous from late-differentiated epidermal cells, the studies should be continued.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OB 6913	76 10 01	DD-DR&E(AR)636	
3. DATE PREV. SUM'Y	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTR*	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3M161102BS02				066	
b. XXXXXXXX	62760A	3A672760A822		01		164	
c. XXXXXXXX	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code)*							
(U) Physical, Chemical Characteristics of Human Stratum Corneum							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
67 07		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:		EXPIRATION:		PRECEDING		76	
b. NUMBER*				FISCAL		2.6	
c. TYPE: Not Applicable		d. AMOUNT:		CURRENT		7	
e. KIND OF AWARD:		f. CUM. AMT.		77		2.6	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Cutaneous Protection Division			
				Department of Dermatology Research			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME* Spencer, T.S., CPT, MS			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Stratum Corneum; (U) Absorption; (U) Permeability; (U) Water; (U) Water Vapor; (U) Chemicals; (U) Persistence; (U) Skin; (U) Human Volunteers							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To define the physical-chemical characteristics of the stratum corneum and its interaction with water, chemicals, ultra violet radiation and environment. These characteristics are fundamental to the etiology of several dermatological disorders caused by exposure of the soldier's skin to the environment and are also applicable to the behavior of topical preparations.							
24. (U) Complete mathematical analysis of hydration data obtained between 1969 and 1972 and obtain data on hydration kinetics to confirm the analysis. Spectroscopic techniques will be employed to assay the denaturing effects of water, ultraviolet radiation, and chemical exposure. Efficacy of protective formulations in preventing denaturation from hydration and ultraviolet radiation will be studied. Stratum corneum from individuals prone versus individuals resistant to disabling dermatoses will be evaluated before and after experimental induction of such disabling conditions.							
25. (U) 76 04 - 76 09 The tyrosine and tryptophan content of stratum corneum was analyzed using quantitative phosphorimetry, and the tryptophan residues were found to be present in the cell lipid-protein matrix as well as in the cell membrane. The water content of stratum corneum appeared to affect both the triplet lifetime of tryptophan emission in stratum corneum as well as the fluorescence spectrum. When compared to the emission spectrum of tryptophan in various solvent, the results indicate that water is interacting with the protein in stratum corneum.							

*Available to contractors upon originator's approval.

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	164	Physical, Chemical Characteristics of Human Stratum Corneum

The following investigations have been conducted under this work unit:

STUDY NO. 6 Determination of Tryptophan Content of Stratum Corneum

STUDY NO. 7 Effect of Water Content on Ultraviolet Emission Properties of Human Stratum Corneum

STUDY NO. 8 Effects of Maturation of the Emission Properties of Stratum Corneum

The tyrosine and tryptophan content of stratum corneum was analyzed by quantitative phosphorimetry, and tryptophan residues were present in the cell lipid-protein matrix as well as in the cell membrane. The water content of stratum corneum appeared to affect the triplet lifetime of tryptophan emission in stratum corneum as well as the fluorescence spectrum. When compared to the emission spectrum of tryptophan in various solvents, the results indicate that water is interacting with the protein in stratum corneum.

Changes in the fluorescence and phosphorescence of maturing stratum corneum indicate that considerable cross-linking occurs; the changes are interpreted to reflect the inverse process of degradation of stratum corneum which results from excessive exposure to water.

BODY OF REPORT

WORK UNIT NO. 164

Physical, Chemical Characteristics
of Human Stratum Corneum

STUDY NO. 6

Determination of Tryptophan Content
of Stratum Corneum

PROBLEM:

The stratum corneum as the barrier between the soldier and his environment is subject to damage when exposed to water (immersion foot) or friction blisters; however, studies of the physical properties of the membrane have been limited to gross observations of the whole cell membranes and matrix protein. Since tryptophan is a potential intrinsic probe for fluorescence and phosphorescence studies of stratum corneum, changes in the emission spectrum due to agents (repellents, medical preparations, or water) could result from interactions with either cell membranes or the protein-lipid matrix within the cell. The mechanical strength and barrier function of stratum corneum depend on the protein matrix; therefore, location of the tryptophan residues exclusively in either the protein or membrane would permit studies of differential damage to the membrane or matrix. Hence, a study was undertaken to determine whether the tryptophan residues were located predominately in the cell protein matrix or the cell membrane.

RESULTS AND DISCUSSION OF THE RESULTS:

Separated stratum corneum membranes were digested in concentrated sodium hydroxide solution for 72 hours, and remnants of the cell membranes were separated by centrifugation. The precipitate was identified as isolated cell membranes by scanning electron microscopy. The supernatant, containing dissolved intracellular material, was analyzed for tryptophan and tyrosine content by quantitative phosphorimetry with a standard addition technique. The tyrosine content was similar to that determined by other techniques, while as much tryptophan as tyrosine appeared to be in stratum corneum. The latter observation is the first estimate of tryptophan content of the intracellular material, because analysis of tryptophan is difficult with the use of standard spectroscopic techniques. The precipitated residue of cell membranes was analyzed spectroscopically; it had a spectrum similar to the spectrum of the intracellular material.

CONCLUSION:

In the present study the tryptophan content of the cell membranes and the intracellular material did not appear to be different.

RECOMMENDATIONS:

Tryptophan as an intrinsic spectroscopic probe of the physical properties of stratum corneum should not be considered as a tool for differentiating effects of topical agents on the cell membrane in contrast to the protein-lipid matrix.

STUDY NO. 7

Effect of Water Content on
Ultraviolet Emission Properties
of Human Stratum Corneum

PROBLEM:

If one desires to understand the effect of water in the penetration of mosquito repellents through skin, transepidermal water loss and the interaction between water and the stratum corneum membrane are important. Tryptophan is an intrinsic probe for ultraviolet emission studies of human stratum corneum. The current study relates the use of the tryptophan to effects of water content on the protein molecules in the stratum corneum. An understanding of water binding in the stratum corneum will permit one to determine the accessibility of skin proteins to hydration, thereby defining possible routes of penetration of water, repellents, or antifungal agents. Knowledge of hydration mechanisms is necessary to an understanding of immersion foot problems in the military, whereas penetration of topically applied formulations is of interest in developing repellent on antifungal formulations to protect the soldier.

RESULTS AND DISCUSSION OF THE RESULTS:

Samples of human stratum corneum were hydrated over salt solutions to provide a water content ranging from 0.01 to 0.8 mg water/mg dry stratum corneum. Two major changes occurred in the characteristic tryptophan emission of the stratum corneum. A spectral peak and a shoulder were observed in the fluorescence emission in dry stratum corneum, whereas this structure was not present in the hydrated samples. This observation was correlated with tryptophan emission in a non-polar solvent where structure was observed in the fluorescence emission as opposed to a structureless band observed in tryptophan fluorescence in a hydrogen-bonding methanol: water mixture. The second observed change in the stratum corneum spectrum was a longer triplet state mean lifetime associated with hydrated stratum corneum in contrast to dry stratum corneum. This also correlated with longer triplet lifetimes associated with tryptophan in hydrogen-bonding solutions compared to non-polar solutions. The two observations indicate that the water of hydration in stratum corneum affects the tryptophan in the protein filaments of the stratum corneum. Hence, protein in addition to the lipid matrix or intercellular areas is directly affected by hydration and it follows that damage to stratum corneum

due to excessive water exposure may be induced by uncoiling of the protein filaments rather than simply a loss of lipid matrix material.

CONCLUSIONS:

Water of hydration directly interacts with the tryptophan residues of the protein matrix of stratum corneum.

RECOMMENDATIONS:

The direct effect of water on the mechanical integrity of the stratum corneum should be considered in situations where excessive water exposure might be observed (i.e. immersion foot, dishpan hands, or afflictions of the groin).

STUDY NO. 8

Effects of Maturation of The
Emission Properties of Stratum
Corneum

PROBLEM:

Since militarily disabling conditions such as miliaria and immersion foot are in part attributed to a breakdown in the mechanical integrity of skin, an understanding of the development of barrier function in stratum corneum might give some insight into the degradation mechanisms of skin. Work at other laboratories has indicated that definite changes occur in neonatal rat skin within 60 hours after birth. Since these changes reflect differences in the interaction of stratum corneum with water, a study of the fluorescence and phosphorescence properties of neonatal rat stratum corneum was undertaken for the purpose of ascertaining some of the mechanism of maturation. Since the fetus lived in an aqueous environment until birth. Presumably, the maturation process might reflect the reverse of the degradation of the membrane by water in future studies of hydration of stratum corneum.

RESULTS AND DISCUSSION OF THE RESULTS:

Neonatal rat stratum corneum specimens were obtained from another laboratory at 0, 10, 30, and 60 hours after birth. Fluorescence at room temperature and fluorescence-phosphorescence at 77°K were determined. In addition, phosphorescence mean lifetimes were measured for each sample. A four-fold decrease was observed in fluorescence intensity between the 0 and 10-hour samples compared to the 30 and 60 hour samples. Moreover, the fluorescence maximum shifted to longer wave lengths with increasing age of the rat corneum. At 77°K the ratio of the tyrosine to tryptophan emission was substantially reduced in the maturation process while the tryptophan lifetime increased between the 0 and 60-hour samples. The reduction

in fluorescence because of increased intersystem crossing intensity and the decreasing tyrosine component in phosphorescence because of increased triplet-triplet energy transfer are both indicative of a cross-linking process occurring in the developing stratum corneum. The increasing tryptophan lifetime implies that the cross-linking has enveloped the tryptophan residues in a more polar environment than was present at birth.

CONCLUSIONS:

A gross alteration occurs in the protein structure of stratum corneum following birth.

RECOMMENDATIONS:

The results should be compared to spectroscopic studies of the effect of denaturing agents such as water which break down the protein structure and integrity of stratum corneum in conditions such as immersion foot.

PUBLICATIONS:

1. Spencer, T. S. and W. A. Akers: Water and the horny layer. (Abstract) of Bronze Medal Award winning exhibit presented at the American Academy of Dermatology, 1975.
2. Spencer, T. S.: Water and the horny layer. J Soc Cosmet. Chem 27:63-72, 1976.
3. Rietschel, R. L., and T. S. Spencer: Correlation between mosquito repellent protection time and insensible water loss from skin. J Invest Dermatol. 65:385-387, 1975.
4. Spencer, T. S. and W. A. Akers: Drug loss from skin by evaporation and penetration. (Abstract) Accepted for presentation at the American Academy of Dermatology, 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISB'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	9. LEVEL OF SUM A. WORK UNIT
76 04 02	D. Change	U	U	NA	NL		
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3M762772A810				006	
EXPERIMENTAL	62760A	3A762760A822		01		167	
C. XXXXXXXX	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Skin Diseases Among Soldiers							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 01		CONT		DA		C. In-house	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
D. NUMBER ^a				76		1.4	
C. TYPE:				FISCAL		.5	
Not Applicable				77		2.0	
E. KIND OF AWARD:				CURRENT		9	
f. CUM. AMT.				77		44	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a : Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME ^a : Letterman Army Institute of Research Cutaneous Protection Division Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS ^a :				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
RESPONSIBLE INDIVIDUAL				NAME ^a : Akers, W.A., COL, MC			
NAME: Canham, J.E., COL, MC				TELEPHONE:			
TELEPHONE: 415-561-3600				SOCIAL SECURITY ACCOUNT NUMBER:			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Applicable				NAME: Greenberg, J.H., LTC, MC			
				NAME: Richey, J.T., DAC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Human volunteers; (U) Occupation; (U) Diagnosis; (U) Skin; (U) Survey; (U) Soldiers; (U) Morbidity							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To determine the type and frequency of potentially disabling skin diseases among soldiers in various environments. To conduct trials of potential preventive and therapeutic agents against the common, disabling dermatoses that afflict military personnel. To develop or improve methods of studying militarily relevant skin diseases under field conditons.</p> <p>24. (U) The proportion of patients with various diagnoses is being tabulated using dermatology clinic visit data from four Army medical centers. Epidemiologic surveys of skin diseases in troop populations are conducted to determine the effect of environment, clothing, and other variables on the frequency and severity of lesions. New diagnostic techniques developed in the laboratory are tested in the field to determine their performance characteristics.</p> <p>25. (U) 76 04 - 76 09 Extensive data collected during epidemiologic surveys of fungal skin infections in American and Colombian Army soldiers were further analysed with computer assistance. This provided new information about the relationship between these infections and environmental stresses, immunological status, and the influence of clothing and footwear. Data were analyzed concerning the field performance of systems for recovering streptococci from normal skin and for recovering staphylococci from skin lesions under adverse conditions. Data were collected concerning the U.S. Army's experience with skin diseases over the past quarter century.</p>							

^aAvailable to contractors upon originator's approval

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DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	167	Skin Diseases Among Soldiers

The following investigations have been conducted under this work unit:

- STUDY NO. 1 Skin Diseases Among Soldiers
- STUDY NO. 3 Dermatophytosis Survey at a U.S. Army Post
- STUDY NO. 4 Skin Infections in Relation to Geographic Environment
- STUDY NO. 5 Recovery of Beta-hemolytic Streptococci from Normal Skin in Field Surveys
- STUDY NO. 6 Recovery of Staphylococcus aureus from Skin Lesions under Field Conditions
- STUDY NO. 7 Antibiotic Resistance of Skin Microflora
- STUDY NO. 8 Treatment of Common Bacterial Skin Infections
- STUDY NO. 9 Statistical Aspects of Studies Involving Skin Diseases

Study No. 1 The ongoing collection of patient visit data from dermatology clinics at four Army Medical Centers was extended to include data from one general hospital at an Army post. Emphasis was placed on time lost from duty because of patient visits. The lost time ranged from 800 to 1900 man days per year per installation.

Study No. 3 Data were analyzed from a fungal skin infection survey of soldiers at Fort Ord, CA. There was no association between atopy (a common inherited allergic condition) and either a history of ringworm or clinical evidence of fungal infection of the body or groin. In addition, there was no evidence of an association between immediate or delayed skin test positivity and either clinical or laboratory evidence of ringworm infection.

Study No. 4 Further analysis of fungal skin infection survey data collected from Colombian Army troops revealed the following:

recruits had little fungal infection of the foot compared to seasoned troops; the rate of infection was highest among troops in the hot-wet climate, lowest in the cool-dry climate, and intermediate in the temperate and hot-dry climates. There were no differences between combat and support troops in rates of infection. The proportion of infections caused by the three ringworm species isolated did not vary according to climate.

Study No. 5 The silica gel swab technique which is useful in recovering streptococci during surveys for "strep throat" and streptococcal skin infections was found to be ineffective in recovering streptococci from normal skin during surveys. There were indications that the reason for failure of the technique in culturing normal skin was the few numbers of organisms present under these circumstances. A system was devised in the laboratory which may make the technique effective in future field tests.

Study No. 6 Two indicator media were specially developed to increase the ability to make a definitive isolation of S. aureus during field studies of skin infections. A paired comparison of the two media under realistic field conditions showed one to be superior in terms of sensitivity, specificity, cost, and ease of preparation. The other medium was easier to read because fewer bacteria grew on it.

Study No. 7 Micrococci (S. aureus and epidermidis) collected from normal and infected skin during the Colombian Army survey were tested for resistance to antibiotics. Preliminary analysis of the data indicates that there is no apparent association between the presence of dermatophyte infections and penicillin-resistant microflora. Likewise, there appears to be no association between the climate in which the various groups surveyed were living and penicillin resistance of the microflora.

Study No. 8 Considerable time and effort were expended in planning and making the preliminary arrangements for a definitive trial of the value of topical antibiotics in the treatment of common bacterial skin infections. The study was not performed because it was disapproved at higher headquarters. This occurred despite the fact that the protocol had been approved by the human use committee of a major medical school, and that permission to conduct the study had been granted by the government of the host country, the pertinent national and local medical authorities, and the representatives of the study population itself.

Study No. 9 Statistical data concerning the Army's experience with skin diseases was collected from standard reports covering the period 1952 to 1972. In a second approach to statistics and skin diseases, preparation was made for publication of a series of articles on the application of statistical and epidemiologic methods in dermatology.

BODY OF REPORT

WORK UNIT NO. 167

Skin Diseases Among Soldiers

STUDY NO. 1

Skin Diseases Among Soldiers

PROBLEM:

There is no morbidity information available which shows time lost from duty for skin diseases. This study was performed in an attempt to collect data of this subject and to analyze them.

RESULTS AND DISCUSSION OF THE RESULTS:

In April 1975, the dermatologic data collection survey was re-initiated at the four Class II Army hospitals that have dermatology residencies (WRAMC, LAMC, FAMC, BAMC). The survey was also started at selected Class I Army hospitals in October 1975. The data collection card was revised to include work loss figures. All active duty visits to dermatology clinics were considered to take two hours unless the examining physician specifically entered a greater number of hours. This number was arrived at by a patient survey but probably it is a low estimate. If a patient was put on quarters, time lost was tallied as 8 hours per day. Examples of time lost for Brooke Army Medical Center on a projected yearly basis show 5,500 man hours lost from duty. At Fort Hood this yearly projected loss of man hours is 15,700.

CONCLUSION:

About 800 man days are lost per year for dermatologic conditions at Fort Sam Houston. At a post where more field training is taking place 1900 man days are lost.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

PROBLEM:

Previous surveys to determine the prevalence and severity of dermatophytosis (ringworm) in CONUS-based military personnel have generally not provided detailed standardized information about the clinical and laboratory findings, nor have they furnished information about the host's (soldier's) immunologic status with respect to dermatophytosis. Lack of this kind of information makes it difficult if not meaningless, to compare results between studies and leaves a void in immunologic knowledge of dermatophytosis.

RESULTS AND DISCUSSION OF THE RESULTS:

A standardized fungal skin infection survey of 188 soldiers at Fort Ord, CA, was conducted in late summer 1974. Complete clinical data were obtained from 165 men, laboratory results from 103, and skin test results from 133. Reductions in numbers were the results of lack of compliance with the survey and loss of laboratory specimens.

Of those for whom results were obtained, 29% had a history of ringworm; 10% had clinical evidence of a body or groin infection (usually minimal) on physical examination of the skin; 30% had scaling in the "target area" of the foot, the right fourth toe web (R4TW); 10% had fissures in the R4TW; 10% had fungal-positive scrapings from the R4TW on microscopic examination; 15% had a positive immediate reaction to commercially-available trichophytin skin testing (greater than a 10mm wheal at 15 minutes); and 9% had a positive delayed reaction to trichophytin (>1mm induration after 3 days). Questionnaire responses regarding atopy (as common inherited condition that may predispose to, or alter the response to, certain kinds of ringworm infections) revealed the following: 4% had a history of asthma; 10% had a history of hay fever; 2% had a history of childhood eczema; and 18% had a parental history of one or more of these conditions. Overall, 25% of the men either had a personal or a parental history of atopy. Also, in response to a self-administered questionnaire, 23% indicated that they were currently using some kind of medication for their skin, usually an over-the-counter preparation for foot or groin dermatosis, or for acne.

There was no evidence of a strong statistical association between atopy and either a history of ringworm or clinical evidence of fungal infection of the body or groin.

Likewise, there was no evidence of a strong association between

immediate or delayed skin test positivity and clinical infection of the body or fungal-positive scrapings from the target toe web. There was no apparent correlation between the presence of physical signs of tinea pedis ("athlete's foot") in the target toe webs and fungal-positive scrapings from the toe webs.

CONCLUSIONS:

These results are in line with other reports indicating that ringworm infections are generally neither common nor severe among military personnel in the United States. The results also indicate that atopy is about as common as would be expected in a young adult population, but that the atopic state does not appear to be strongly associated with fungal skin infections in soldiers, at least in the United States. The skin test responses did not appear to be predictive, which may reflect the long-suspected non-specificity of the antigens present in commercially-available trichophytin or the lack of value of skin testing per se.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

STUDY NO. 4

Skin Infections in Relation to
Geographic Environment

PROBLEM:

Climate and hygiene are both thought to be of major importance in determining the frequency and severity of common fungal and bacterial skin infections. Strong evidence has previously been adduced for this in the case of infections caused by streptococci (bacteria), but no comparable strong evidence has been available for fungal infections caused by dermatophytes (ringworm fungi) and yeasts. Such information is needed in order to develop rational approaches for prevention of fungal skin infections, which are a major cause of time lost from infantry duty in tropical zones.

RESULTS AND DISCUSSION OF THE RESULTS:

Further analysis of data collected during a survey of 1042 Colombian Army soldiers in late 1974 showed that the highest rate (70%) of fungal infections involving the body or groin was in the hot-wet climate, and the lowest rate (7%) was in the cool-dry climate. Rates of infection in the hot-dry, temperate-wet, and temperate-dry climates ranged between 17 and 27% with no clear pattern emerging in relation to climate.

Recruits differed markedly from seasoned troops (6 months service or more) in rates and severity of infection, especially of the feet, and therefore findings were stratified according to length of military service. Results based on the 793 men with at least 6 months service were used to establish relationships because this length of service was judged to provide a relevant period of exposure to military life and its associated exposures to communal living facilities, standard items of clothing and footwear, and a given type of climate.

Regardless of body area considered, as long as it was above the soles of the feet, the rate of infection was consistently highest among troops in the hot-wet climate, lowest in the cool-dry climate, and intermediate in the other three climates. The legs, buttocks, and waist area showed especially pronounced elevations in fungal infection rate among men in the hot-wet climate compared to men elsewhere.

Severe, widespread infections were common in the hot-wet climate and nonexistent in the cool-dry climate. There was a low and homogeneous frequency of these infections in the three intermediate climates.

Except for new recruits, who had virtually no signs of infection of the soles of the feet or the toe webs, every man had at least some minor scaling or other sign of fungal infection of the bottoms of feet. Three physical signs of foot infection--maceration, redness, and denudation--could be related to climate; but another two--scaling and skin cracks--could not. The pattern was not consistent from sign to sign and was much less striking than the findings associated with infection elsewhere on the body.

Cultures of scrapings taken from the "target area" of the foot (right fourth toe web) showed that Candida albicans was much more common in the hot-wet geographic region than in the other climates; otherwise the results of culture showed no clear pattern according to climate.

The survey results suggest: (1) fungal skin infections involving the body and groin are strongly influenced by both heat and humidity; (2) foot infections are not similarly affected by climate but instead relate to length of service; and (3) other than C. albicans, the frequency of infecting fungal species is affected by climate only slightly.

Only subtle differences between infantry and support troops at each location were detected. These findings indicated that occupationally related differences in physical activity, outdoor

exposures, and levels of hygiene were unimportant as determinants of disease frequency among the Colombian Army soldiers. This contrasted with the situation among U.S. troops. This high rate was directly related to the infantry men's frequent exposures to swamp and paddy water.

CONCLUSIONS:

The relationship between foot infections and length of military service could represent increased transmission of fungi under communal living conditions, but probably it represents the effects of wearing heavy occlusive leather boots.

As suggested in previous work, occlusion of the skin by sweat-dampened clothing or footwear seemed to be the common environmentally related mechanism for inducing susceptibility to fungal skin infections. The high rates of infection in certain climates, or when wearing certain footwear, cannot be reasonably explained by such things as immunologic susceptibility due to atopy. They do indicate the over-riding importance of such environmentally related factors as occlusion of the skin by water or sweat-dampened clothing.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

STUDY NO. 5

Recovery of Beta-hemolytic
Streptococci from Normal Skin
in Field Surveys

PROBLEM:

Beta-hemolytic streptococci are by far the most common cause of the "jungle sores" that plague infantrymen in the tropics. Normal-appearing skin may harbor these bacteria for weeks before they invade at the site of a minor scratch or other break in the skin. Since this has major implications for developing methods to prevent these infections, it is important to determine whether skin colonization is a world-wide phenomenon (especially in the tropics), or whether it is a more isolated phenomenon primarily confirmed to certain groups in temperate countries where it was first discovered. A major obstacle to determining the answers in remote and underdeveloped tropical areas is the lack of uncumber-

some failure-proof field methods for recovering streptococci from normal skin. The existence of a good field method silica gel dessicated swabs for recovering streptococci from throats or infected skin lesions suggested that the method might be adaptable to culturing normal skin. The silica gel dessicated swab system was therefore compared to direct plating on fresh media in the field during a skin infection survey of Colombian Army soldiers (Study No. 4).

RESULTS AND DISCUSSION OF THE RESULTS:

By using the paired-swab technique in a survey of 342 men, beta-hemolytic streptococci were recovered from the normal ankle test sites of 17 men by direct plating, but from no men by use of the silica gel dessicated swab method. Streptococci were recovered from skin lesions in 23 of 84 men by direct plating, and from 19 by using the silica gel dessicated swab system. These results indicated that the silica gel system was approximately as effective as direct plating in sampling infected lesions but was completely ineffective in sampling normal skin.

The difference in recovery rates between swabs taken from normal sites and swabs from lesions suggested that the success of the silica gel system is dependent on density of the inoculum. To see if this was true, serial tenfold dilutions of a broth culture of a representative streptococcus were prepared, and swabs inserted into the diluted cultures before use in the silica gel system. In tests conducted at weekly intervals after insertion of the swabs into the silica gel packets, at least 10 colony-forming units (cfu) of bacteria per ml of broth was required to survive dessication during the first 4 weeks. Fewer organisms (10 cfu per ml) were required when the swabs were incubated in broth prior to dessication. Recovery of streptococci decreased after the first 4 weeks, and, at 8 weeks, 10 times as many organisms were required to yield positive cultures than at 4 weeks.

CONCLUSIONS:

These observations indicate that, during normal skin site surveys, incubation of swabs in a selective enrichment broth may allow streptococci obtained from uninfected skin to increase in numbers to the point where they can survive dessication in silica gel. This adaptation, if it performs adequately in future field test, will expand the use of the silica gel dessicated swab system to culture of normal skin for beta-hemolytic streptococci.

RECOMMENDATIONS:

The suggested adaptation of the silica gel dessicated swab system should be tested in future field studies.

PUBLICATIONS:

None.

STUDY NO. 6

Recovery of Staphylococcus aureus
from skin lesions under field
conditions

PROBLEM:

Staphylococci are a common cause of bacterial skin infections among military personnel. Field studies of these infections in remote and underdeveloped parts of the world are hampered by the lack of a method for recovering staphylococci from skin lesions which is comparable to the silica gel swab system for streptococci. One possible approach to this problem is to develop a culture medium which is highly selective so that it does not become contaminated if used in the field and which contains an indicator system to distinguish colonies of staphylococci from colonies of other bacteria. This way, results could be obtained in the field by use of portable field incubators, and there would be no need to subculture colonies in the field and return them to a base laboratory for positive identification. Two such media were developed independently --- one at LAIR and another at the University of Miami, Fl (under Army contract). These were compared during a skin infection survey of Colombian Army soldiers (Study No. 4). The LAIR medium was designated MSE (mannitol-salt-egg yolk) and the Miami medium, PYM (polymixin B-yeast-mannitol).

RESULTS AND DISCUSSION OF THE RESULTS:

Paired swabs from 90 infected skin lesions were cultured on PYM and MSE. An additional swab was plated on blood agar. Sixty four (71%) of the 90 lesions were positive for S. aureus as determined by coagulase confirmation testing of all representative colony types from each of the three media. These results were used as the standard against which the performance of each of the new media was compared.

By using the purple-to-yellow color indicator system in PYM, there were 10 false negatives and one false positive; the sensitivity was 84% and the specificity was 96%.

MSE incorporated two indicator systems: one a color change from pink to yellow, and the other the formation of an opaque precipitate around the colony. The two indicator systems were independent of one another, and therefore results were recorded separately.

By using the pink-to-yellow color indicator system in MSE, there were 3 false negative and 7 false positives; the sensitivity was

95% and the specificity was 73%. With the precipitate indicator system, there were 8 false negatives and no false positives; the sensitivity was 88% and the specificity was 100%.

These results suggest that, if PYM is compared to the egg yolk (precipitate) component of MSE, there is little to choose between the two. The small observed differences in specificity and sensitivity can easily be attributed to chance (sampling error). However, the mannitol (color change) component of MSE was substantially more sensitive but less specific than PYM, and these differences were statistically significant.

By combining the results obtained from the two indicator systems, MSE produced essentially the same information as mannitol results alone; thus, no diagnostic advantage was gained. However, if one coagulase-tested colonies from only those plates which showed mannitol-positive, egg-yolk-negative reactions, specificity with MSE would be increased from 73 to 100% with no loss of sensitivity. Thus, maximum sensitivity and specificity could be obtained by using MSE, provided that the colonies from a minority of mannitol-positive plates were subcultured for later coagulase-testing at a base laboratory. Although PYM was comparably specific, no such increase in sensitivity could be brought about by additional testing since the medium was so highly selective that it inhibited the growth of some staphylococci.

CONCLUSIONS:

MSE is a great deal cheaper and easier to prepare than PYM, and thus confers the additional advantages of economy and convenience. The chief advantage of PYM was that, because of less luxuriant growth of organisms, it was easier to read than MSE.

RECOMMENDATIONS:

MSE should be used in future field studies concerned with detecting the presence of S.aureus in skin lesions.

PUBLICATIONS:

None.

PROBLEM:

Ever since the introduction of antibiotics into medical use about 30 years ago, it has been noted that a number of potentially harmful bacterial species, notably Staphylococcus aureus, could develop antibiotic resistance. Development and spread of antibiotic resistance among medically important bacteria has been largely attributed to alleged profligate over-prescription of antibiotics. However, this does not explain the increasingly widely noted phenomenon of antibiotic resistance where there seems to be little, if any, overt ecologic pressure because of widespread use of antibiotics. In fact, penicillin-resistant strains of S. aureus have been found as often, and in nearly as great a proportion, on the skin of people living in remote parts of the world where antibiotics are scarce as it has been found in infected lesions in patients and hospital personnel. These findings have important implications for antibiotic prescribing practices in various parts of the world where military personnel may be stationed. They may serve as a clue to some of the factors influencing the skin microflora which are responsible for producing such militarily important conditions as "jungle sores." A possible explanation for the high proportion of penicillin-resistant staphylococci on the skin of persons with little or no contact with medicinal sources of penicillin is the presence of penicillin-producing microorganisms in the environment. A number of microorganisms, among them dermatophytes (ringworm fungi), produce penicillin or penicillin-like substances. It is conceivable that they could be providing the ecologic pressure responsible for the widespread occurrence of penicillin-resistant S. aureus. If this were true, it might be expected that persons with ringworm infections would have a higher proportion of penicillin-resistant micrococci (S. aureus and S. epidermitis) on their skin than persons without these infections, and that persons living in hot, humid tropical areas where fungal infections are rife would also have a higher proportion of penicillin-resistant micrococci on their skin than persons living in temperate or cool climates where fungal skin infections are less common. These hypotheses were tested by using specimens and data collected during a skin infection survey of Colombian Army soldiers (Study No. 4).

RESULTS AND DISCUSSION OF THE RESULTS.

Data are available from the 342 soldiers whose ankle skin (normal sites) was routinely cultured for streptococci and micrococci. Additional data came from the 90 soldiers whose skin lesions were

cultured for bacteria. Analysis of the data is not yet complete, but early indications are that there is no apparent association between the presence of dermatophyte infections and penicillin-resistant microflora, nor does there seem to be an association between climate and resistant microflora.

Bacillus species of bacteria were also commonly recovered from the normal skin of the ankles, and these were also studied because they are known antibiotic producers. Again, no associations were found between their presence and the presence of antibiotic-resistant micrococci during a preliminary analysis of the data.

CONCLUSIONS:

Further analysis of the data is required before any definite conclusions can be reached. If the results of the preliminary analyses hold up under further scrutiny, a major reexamination of some emerging concepts in the field of microbial interactions on the skin would be in order. Also, no plausible theory would exist for the high prevalence of penicillin-resistant S. aureus in communities and parts of the world where there is little or no medicinal use of penicillin.

RECOMMENDATIONS:

The data will be analyzed further.

PUBLICATIONS:

None.

STUDY NO. 8

Treatment of Common Bacterial
Skin Infections

PROBLEM:

The most common form of bacterial skin infection worldwide is streptococcal pyoderma. Streptococci are the predominant cause of so-called "jungle sores" which plagued American forces fighting in the tropics in World War II and again in Vietnam. It is now known that these sores can be successfully treated by one injection of a long-acting form of penicillin or by a 10-day course of erythromycin by mouth if the patient is allergic to penicillin. Although successful, this form of treatment is not as appropriate for small or early lesions as it is for large well-established lesions because of the pain associated with the injections, compliance problems in taking pills for 10 days, and the possibility of serious allergic reactions to systemically administered antibiotics.

Topical antibiotics such as neomycin or bacitracin in a cream or ointment base have been promoted for some 20 years as the treatment of choice for small cuts, scratches, or bacterial skin infections. There is good evidence that topicals are not nearly as effective as systemic antibiotics for large streptococcal skin infections called "ecthyma," but the evidence is unclear as to whether or not they may confer any significant benefit for lesions. Since topical antibiotics are in widespread use for skin infections, especially in the Armed Forces, it would seem advisable, if not imperative, to determine in a rigorous scientific manner whether or not they are beneficial.

A clinical trial of a topical antibiotic (in this case neomycin, the most widely used agent) for the treatment of pyoderma was conceived by members of LAIR and a civilian contractor at the University of Miami, FL. Arrangements were made to conduct the study in a group of school children living in a tropical village in a Central American country. A liaison visit and preliminary surveys had established that these children were at high risk to developing pyoderma and that the population was freely willing to participate in the study. Half the children enrolled in the trial were to receive systemic antibiotics for their sores, and half the topical antibiotic, with one quarter receiving both antibiotics and one quarter no antibiotics (2x2 factorial design). The lesions were to be classified clinically in a standard manner so as to be able to determine where the "cutoff point," if any, was between lesions which would be benefitted by application of a topical antibiotic and those which would not. Approximately 200 children with sores would be available for the study.

RESULTS AND DISCUSSION OF THE RESULTS:

This study was not performed because final permission was not granted to conduct it. Among the reasons for withholding final permission were: (1) the value of topical antibiotics in the treatment of pyoderma was not considered a "burning issue" because penicillin was known to be effective in the treatment of "reasonably" severe (sic) pyoderma; (2) it was not apparently appreciated why children should be "subjected to the pain" of a benzathine penicillin injection; (3) it was also apparently not appreciated why it would be appropriate for children to serve as test subjects to provide militarily relevant answers; and (4) relations between the United States government and the host country were too "sensitive" to permit a study of this kind.

While it may be agreed that these appear to be valid objections, it is important if not imperative that they be seen in context. Prior to requesting final permission to conduct the study, the protocol had been approved by the Ministry of Health of the host

country and by the Human Use Committee at the University of Miami. It had also met with the approval of the authorities of the medical school in the host country and the approval of the village leaders.

It may be noteworthy that nobody can have the vaguest idea of what the definition of "reasonably severe" pyoderma is because the degrees of severity of this disease have never been classified and defined in an appropriate manner. For this and other reasons, it is quite possible that children the world over are needlessly receiving painful and possibly dangerous injections of penicillin for cases of pyoderma which might be successfully treated by a topical antibiotic alone.

The propriety of children being research subjects in this instance is based on several considerations. Probably the most cogent argument is that, with the exception of ground combat troops in tropical war zones, children in the tropics are at extremely high risk to developing infections and therefore are the principal sufferers from the disease.

CONCLUSIONS:

Preventive measures are inadequate at best, and various social and economic considerations dictate the systemic antibiotics will seldom be available for most cases of pyoderma. Whether it is reasonable or helpful for children (or soldiers) in the tropics or elsewhere to use topical antibiotics for treatment of pyoderma can only be determined by well-conducted trials in appropriate populations at risk.

RECOMMENDATIONS:

A trial of the type described should be conducted whenever permission is granted and the needed resources, including experienced investigators and a suitable patient population, are available.

PUBLICATIONS:

None.

STUDY NO. 9

Statistical Aspects of Studies
Involving Skin Diseases

PROBLEM:

Many studies of skin diseases, including those that are militarily relevant, are essentially statistical in nature; that is, they depend on numbers to describe or analyze what is going on with respect to the disease. Prominent among these studies are

epidemiologic surveys, clinical trials, evaluation of diagnostic test, and historical documentation of the importance of certain diseases. Many of the investigators conducting these studies have only a limited knowledge of statistical methods, and little access to meaningful statistical consultation. Consequently, it is commonplace to find serious errors in the dermatologic literature, and equally serious misconceptions in the minds of a number of investigators who are otherwise well qualified to produce valid work. We have sought to overcome this problem by approaching it two ways (1) by describing and analysing the U.S. Army's experience with skin diseases and (2) by preparing review articles that show by precept or example how to deal statistically with skin diseases.

RESULTS AND DISCUSSION OF THE RESULTS:

Statistical reports and data (1952 to 1972) from the Office of the Surgeon General, DA, were collated and analyzed. Among the findings were that the rate of admissions for skin diseases among Army personnel in CONUS over the period 1952 to 1972 dropped 80%; that skin diseases were the leading cause of outpatient visits in Vietnam and the third leading cause of hospitalization for disease; and that a regular "summer hump" of admissions for skin diseases occurred during the 1950s which had disappeared by the 1960s. The rate of admissions and outpatient visits for skin diseases in soldiers is geographically dependent; it is lowest in Alaska, intermediate in Europe and the United States, and highest in the Caribbean, Vietnam, and other tropical areas.

In the second approach to statistics and skin diseases, material was gathered from a variety of published sources for inclusion in a series of articles on the application of statistical and epidemiological methods in dermatology. The subjects completed to date include experimental design of clinical trials, sample sizes (numbers of patients) required in clinical trials, and descriptive epidemiology of skin diseases.

CONCLUSIONS:

None.

RECOMMENDATIONS:

None.

PUBLICATIONS:

1. Allen, A.M., and D. Taplin, Epidemiology of cutaneous mycoses in the tropics and subtropics; Newer concepts. Pan Amer. Health Org. Sci. Pub. No. 304:215-223, 1975.

2. Ad Hoc Committee on Use of Antibiotics in Dermatology (Akers, W.A., A.M. Allen, and others). Systemic Antibiotics for Treatment of Acne Vulgaris: Efficacy and Safety. Arch. Dermatol. 111:1630-1636, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OC 6913	76 09 30	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISB'N INSTR*	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H.Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62760A	3A762760A822		01		168	
b. XXXXXX							
c. XXXXXX	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code)*							
(U) The Effects of Prolonged Water Exposure on Human Skin (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 04		76 09 30		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER:				76		1.0	
c. TYPE: Not Applicable				FISCAL		3	
d. AMOUNT:				77		0	
e. KIND OF AWARD:				CURRENCY		0	
f. CUM. AMT.				77		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				Cutaneous Protection Division			
RESPONSIBLE INDIVIDUAL				ADDRESS* Department of Dermatology Research			
NAME: Canham, J.E., COL, MC				Presidio of San Francisco, CA 94129			
TELEPHONE: 415-561-3600				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
21. GENERAL USE				NAME* Grekin, David, MAJ, MC			
Foreign Intelligence Not Applicable				TELEPHONE: 415-561-5485			
				SOCIAL SECURITY ACCOUNT NUMBER:			
				ASSOCIATE INVESTIGATORS			
				NAME: Allen, A.M., LTC, MC			
				NAME: Spencer, T.S., CPT, MSC POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Skin; (U) Foot; (U) Dermatophytes; (U) Water; (U) Human Volunteer (U) Immersion Foot; (U) Cold Injury;							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To determine the clinical, physical, biochemical and microbiological changes that result from prolonged exposure of the skin to water; and to develop effective methods of preventing warm water immersion injuries. Practical effective preventive measures could prevent large numbers of "tropical immersion foot" casualties from occurring among ground combat troops operating in warm, wet areas, such as swamps paddies and flooded plains, as well as from accumulated sweat while wearing waterproofed footwear.</p> <p>24. (U) To develop model water immersion injuries in volunteers; to test the effects of changes in pH and other variables such as soaps and creams, in such model injuries; prophylactic agents and modalities will be tested and evaluated.</p> <p>25. (U) 76 04 - 76 09 The pertinent literature has been reviewed and a study protocol and two pilot study protocols have been written. Two allegedly waterproof sun screens were evaluated using volunteers and a small prolonged water immersion field study was conducted using volunteers. The work covered by this work unit is being merged into work unit 002, beginning 76 10 01.</p>							

*Available to contractors upon originator's approval.

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DD FORM 1498

1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	01	Internal Medicine
WORK UNIT NO.	168	The Effects of Prolonged Water Exposure on Human Skin

Warm-climate water immersion injuries produce high rates of disability in soldiers exposed for prolonged periods to wet terrain. An experimental model has been developed for the injury to the sole of the foot. A more serious form of injury involves the top of the foot and the lower leg. Little is known concerning the pathogenesis of this type of injury and there is some confusion about the actual clinical description of this entity. A special study involving three volunteers was done in the Sacramento River delta to reproduce the warm water tropical immersion foot that afflicted soldiers in Vietnam and the Philippines. The experiment was successful.

A pilot study showed two standard sunscreens incorporated into a waterproof polymer vehicle offered protection against sunburn after 60 minutes of swimming. One gave protection for 80 minutes.

BODY OF REPORT

WORK UNIT NO. 168

The Effects of Prolonged Water
Exposure on Human Skin

PROBLEM:

Injuries to the feet known as "warm water immersion foot" and "tropical immersion foot" (paddy foot), accounted for up to 50% of the total man-hours lost among troops operating in the flooded terrain of the Mekong Delta of South Vietnam. Similar statistics were reported in United States troops fighting to retake the Philippines. Additionally, a potential problem exists in Arctic and other cold climates where combat troops may be forced to wear insulated boots for prolonged periods. Waterproof footwear retains sweat so that, in effect, the foot is continuously immersed in warm water. There is no known method of preventing these injuries short of reducing the duration of exposure to water. This has the serious disadvantage of being a major limiting factor in conducting ground combat operations in wet or cold terrain. A convenient, easily controlled method of experimentally inducing warm water immersion foot in volunteers has recently been developed and may prove useful in future study of this injury. However, no model exists for the more severe form of injury, tropical immersion foot. In fact, no investigator has ever seen a fresh case of tropical immersion foot in the field.

RESULTS AND DISCUSSION OF THE RESULTS:

The pertinent literature concerning the immersion foot syndromes was reviewed. It became apparent that no good clinical description of tropical immersion foot existed and that without a better understanding of the clinical manifestations further study would be difficult. There is even some question as to whether or not the entire problem lies solely with water exposure. The occlusive effect of the boot needs to be investigated. A pilot study was conducted in the Sacramento River Delta in swampy terrain similar to the Mekong River Delta area. Three volunteers spent 96 hours walking in water and mud and sleeping with their feet in water.

A pilot study of two new sunscreens incorporated in waterproof polymers was conducted. One sunscreen offered protection against sunburn after 60 minutes of water immersion and the other offered protection after 80 minutes of water immersion. These waterproof polymers may be useful to protect the skin from the effects of water, but further study is necessary.

CONCLUSIONS:

Warm water immersion foot can occur in California as well as the tropics. Polymers offer a method to make pharmaceutical agents withstand water immersion and rain.

RECOMMENDATIONS:

Work should continue on the effects of prolonged water immersion on human skin because of its military importance. A large scale, paired comparison field study should be performed to determine the role of the boot in tropical immersion foot.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OA 6370	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISB'H INSTN*	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3M762772A811		00		004	
XXXXXXXXXX	62760A	3A762760A822		02		083	
XXXXXXXXXX	CARDS 114f						
11. TITLE (Precede with Security Classification Code)*							
(U) Military Food Hygiene							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*							
006500 Food, 007800 Hyg. & Sanitation, 016800 Toxicology, 010100 Microbiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 04		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		76 6.0 241	
B. NUMBER* Not Applicable				FISCAL		7T 1.5 69	
C. TYPE:				YEAR		CURRENT 77 6.0 239	
D. KIND OF AWARD:				F. CUM. AMT.			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS* RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415 561-3600				NAME* Letterman Army Institute of Research Food Hygiene Division ADDRESS* Department of Nutrition Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME* Fowler, James L., COL, VC TELEPHONE: 415 561-2963 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: Fruin, John T., LTC, VC NAME: Taylor, Stephen L., DAC POC:DA			
21. GENERAL USE				22. KEYWORDS (Precede EACH with Security Classification Code)			
Foreign Intelligence Not Considered				(U) Wholesomeness; (U) Data Collection; (U) Microbiological Limits; (U) Safety; (U) Foodborne Diseases; (U) Food Contaminants; (U) Methodology			
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) Submits recommendations to TSGO concerning the workability and suitability of analytical techniques contained in specifications. Recommends food safety standards to TSGO for DoD procured subsistence. Compiles, analyzes, and assimilates into reports microbiological and toxicological data generated internally and received from DoD food analysis laboratories. Develops and evaluates new methodology in the areas of food toxicology, microbiology and virology analyses. Provides information and cooperation with regulatory agencies in the area of food safety and wholesomeness.							
24. (U) DoD directed research in the area of toxic materials produced by microorganisms and insects will be initiated. Efforts will be centered on compounds known or suspected to be toxic with the eventual goal of conducting animal feeding studies. The investigation of selective media and confirmatory techniques for <i>Bacillus cereus</i> will be conducted. The effect of microwave cooking on pathogenic microbial populations will be studied.							
25. (U) 76 04 - 76 09 The study of the characteristics of <i>Clostridium perfringens</i> isolated from foods will continue. An investigation of the microbial flora of comminuted turkey meat was undertaken and nears completion. The results of a study of laboratory analysts' speed and accuracy of counting agar plates are being prepared in final form. A qualitative and quantitative study of the microbial flora of freshly prepared comminuted beef, textured soy protein, and both items after blending (soy protein fortified ground beef) and after 7 days storage has been initiated. Procedures for the isolation and characterization of viruses in foods have commenced and will be continued. The search for effective cryoprotective agents for <i>C. perfringens</i> will go on. The collection of microbiological data reported by DoD food analysis laboratories has continued. Liaison with DoD, federal, state, and non-governmental agencies in the area of food hygiene has been maintained.							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A822 Military Internal Medicine
TASK NO. 02 Nutrition and Wholesomeness Support
for DoD Food Program
WORK UNIT NO. 083 Military Food Hygiene

The following investigations have been conducted under this work unit in the current fiscal year:

- STUDY NO. 11 Investigations of Microbiological Methodology Performed in Conjunction with the Chapter Chairman, Agar Plate Method, for Development of the 14th Edition of Standard Methods for the Examination of Dairy Products
- EXP-4 A Statistical Comparison of Selected Standard Methods Agar Plate Count Limits and Counting Efficiency of Selected Plate Count Ranges by Experienced Analysts
- STUDY NO. 12 Investigations into the Microbiological Quality of Food Items Purchased by DoD for the Purpose of Establishing Tentative Microbiological Guidelines for Military Subsistence
- EXP-3 A Survey of the Microflora of Fresh and Frozen Seafood with Special Emphasis on the Isolation and Identification Procedures for *Vibrio parahaemolyticus*
- EXP-5 Microflora of Ground Turkey
- EXP-6 A Survey of the Microbial Flora of Soya Protein Extended Ground Beef and Its Components
- STUDY NO. 13 Investigations into the Use of Group D Streptococci as Indicators of the Hygienic Quality of Frozen Food Products
- STUDY NO. 14 Typing *Clostridium perfringens* Isolated from Foods
- STUDY NO. 15 The Effect of Cryoprotective Agents in the Survival of *Clostridium perfringens* Type A Vegetative Cells in Selected Meat Products After Freezing and Thawing

- STUDY NO. 16 Computerized Data Collection Program in Food Microbiology
- STUDY NO. 17 Investigations into Potential Toxicological Problems Associated with Food Items Purchased by DoD: Identification of Hazardous Substances, Evaluation of Food Safety, Alleviation of Toxicological Problems and Establishment of Tentative Toxicological Guidelines for Military Subsistence
 - EXP-1 Histamine Production by Food Spoilage Microorganisms and Development of an Analytical Method for Detection of Histamine in Food Products
 - EXP-2 The Toxicity of Histamine and Other Biogenic Amines as Related to Food Poisoning, the Presence of Inhibitors of Histamine-Metabolizing Enzymes in Foods, Dietary Conditions, and Other Gastrointestinal Factors
- STUDY NO. 18 Investigations into Methodology for Detecting and Identifying Viruses in Selected Department of Defense Subsistence Items
- STUDY NO. 19 Investigations into Potential Insect Induced Toxicological Problems in Food
 - EXP-2 Benzoquinones from the Secretory Glands of *Tribolium confusum* and *Tribolium castaneum* - Assay, Reactions, Effects, and Toxicity

Study 11, Experiment 4. The accuracy of laboratory personnel in estimating the microbiological population in a food item is extremely important from both regulatory and economic aspects. Consumer pressure is causing regulatory agencies to develop and adopt microbiological criteria for an increasing number of food items. Interest has developed on methods to decrease the time and expense required in performing standard method plate counts on food items. Controlled studies are required to determine statistically the speed and accuracy of laboratory analysts as well as automated counting devices.

Study 12, Experiment 3. Microbiological analyses of 597 units of seafood, representing 11 distinct types, were performed. Extreme variability was found in the total aerobic plate counts (and in total coliform count). No *Salmonella* or *Vibrio parahaemolyticus* isolations were made; however, *Staphylococcus aureus* and *Clostridium perfringens* were isolated from 7.9% and 2% of the units, respectively.

Study 12, Experiment 5. Due to economic reasons, interest has focused in recent years on the use of comminuted meats such as lamb, veal,

pork, and turkey as alternatives to ground beef in the soldier's diet. Unfortunately, the microbial flora of these products has not been well characterized. In this study, fresh, frozen, and preseasoned comminuted turkey meats were analyzed and microbiological characterization was accomplished.

Study 12, Experiment 6. The use of isolated soy protein as an extender for ground beef has been shown to effect considerable economic savings and the military services have expressed interest in its use in order to stretch their food dollar. With the addition of soy protein to ground beef, however, a product with altered microbial considerations may have been created. Investigations are being performed in this study to characterize soy extended ground beef microbiologically and to compare the findings to those obtained from ground beef alone.

Study 13. In regulatory food microbiology, the presence of organisms, such as *Escherichia coli* and the enterococcal portion of the Group D streptococci, are commonly interpreted as evidence of fecal contamination in the finished product. Most food microbiological criteria are based on the presence or absence of *E. coli*; however, several authorities in the food microbiology field have found that this organism is easily injured by freezing and thawing. It has been speculated that the Group D streptococci are better indicators of fecal contamination in a frozen food product. Using experimentally inoculated foods, it was shown that both *E. coli* and the Group D streptococci survive freezing and thawing as indicated by colony growth on non-selective culture media. However, when the selective media recommended for primary isolation of the respective organisms were employed, *E. coli* was not recovered while the enterococcal portion of the Group D streptococci was completely recovered.

Study 14. Strains of *Clostridium perfringens*, originally obtained from foods, were isolated in pure cultures. After isolation they were typed by the mouse neutralization technique, cultures lyophilized, and catalogued into the Food Hygiene culture collection for future use.

Study 15. It has been postulated that *Clostridium perfringens* may be the causative agent in many of the foodborne illnesses in which the etiologic agent is undetermined. This reasoning is based on the fact that vegetative *C. perfringens* cells do not survive freezing very well, and that many food specimens suspected of causing foodborne illnesses are shipped to public health laboratories in the frozen state. Several chemicals are being screened to determine their cryoprotective abilities for *C. perfringens*. Those chemicals showing protective abilities will be added to various food specimens containing known levels of *C. perfringens*. The food specimens will be frozen, stored for varying lengths of time, and the survival rate of *C. perfringens* determined. The chemical demonstrating the best cryoprotective ability will be determined.

Study 16. A Computerized Data Collection Program in Food Microbiology has been conducted by this laboratory since 1972. It was initially designed as a continuing program to establish a sound data base from which to recommend microbiological guidelines for military subsistence. The program was funded for three years under another work unit (3A161101A91C).

During Fiscal Year 1976, the 1974 file was reported, and the 1975 file was prepared for publications. Calendar year 1976 data are current. On the basis of data contained within the file, recommendations for microbiological guidelines for comminuted beef items and prepared sandwiches have been made. The validity of microbiological specifications for delicatessen salads have been substantiated, and certain problem areas in military food hygiene identified. The program has proven flexible, adaptable, and extremely useful.

Study 17, Experiment 1. Histamine, a product of microbial growth, is intimately associated with foodborne illnesses, particularly scombroid fish poisoning. A relatively sensitive and specific analytical method to determine histamine levels in foods, as well as a means to determine the capabilities of different species of microorganisms to produce histamine needs to be developed. Preliminary research is being conducted under this study.

Study 17, Experiment 2. The mechanism of oral histamine toxicity remains unknown. Three enzymes in the gastrointestinal tract are involved in histamine metabolism and detoxification. The possibility of foodborne inhibitors of these enzymes needs to be explored as a possible explanation of histamine toxicity. The influence of other dietary and gastrointestinal factors on histamine toxicity will also be studied.

Study 18. Procedures for isolation and identification of viruses in military subsistence items are being established. Selected food items are being analyzed for the presence of viruses. On the basis of initial results, research into specific areas or products will be initiated.

Study 19, Experiment 2. Common flour beetles secrete benzoquinones that scientific evidence would suggest are toxic. Further investigations of the acute and chronic toxicity of these benzoquinones coupled with research into better analytical methods for their detection and reactions of biochemical significance are being conducted under this study.

BODY OF REPORT

WORK UNIT NO. 083 Military Food Hygiene

TASK NO. 02 Nutrition and Wholesomeness Support
for DoD Food Program

STUDY NO. 11 Investigations of Microbiological Methodology Performed
in Conjunction with the Chapter Chairman, Agar Plate
Method for the Development of the 14th Edition of
Standard Methods for the Examination of Dairy Products

EXP-4 A Statistical Comparison of Selected Standard Methods
Agar Plate Count Limits and Counting Efficiency of
Selected Plate Count Range by Experienced Analysts

PROBLEM:

A number of Department of Defense (DoD) specifications for the procurement of subsistence items prescribe microbiological standards. The uniform application of these standards is contingent on the use of universally applied, acceptable laboratory procedures which are provided in the specification and referenced in Standard Methods for the Examination of Dairy Products (SMEDP) and Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC).

To estimate the bacterial population of a food item using standard methods agar (SMA) plates, the procedures specify the counting of plates having between 30 and 300 colonies. A reduction in the count range of 30-300 to 20-200 or 10-100 would save a great deal of laboratory analysts' time. If changing the count range did not decrease accuracy, a reduction in count range would be welcomed by laboratory managers and analysts alike.

The standards of accuracy for analysts is to reproduce their own counts within 5% and to reproduce the counts of others within 10%. The method for evaluating accuracy, however, has not been directed. Thus, a meaningful and uniform application of this standard is impossible.

RESULTS AND DISCUSSION OF RESULTS:

Six analysts with varying levels of experience in performing SMA plate counts, were given a series of several hundred SMA plates to be counted. Each of the plates was also photographed. By marking and counting the colonies on the photograph as they were verified against the SMA plates, a true plate count was established.

The mean of the counts of the six analysts was calculated for each plate (mean count) and compared to the true count. The mean count was within 5% of the true count on 62, 76, 81, and 84% of the plates in the

count ranges of 10-100, 20-200, 30-300, and 40-400, respectively. Similarly, the mean count was within 10% of the true count for 89, 96, 97, and 98% of the plates. The mean count was found to be a measurably consistent low estimator of the true count over a range of 0-<400 colonies per plate. The mean count was found to be within 10, 7, 6, and 7% of the true count on 95% of the plates in the count ranges of 10-100, 20-200, 30-300, and 40-400, respectively.

The specialized photographic support required to produce a true count by this method is not readily available to food microbiological testing laboratories. Thus, the use of photographs to establish a true count is not practical. A suitable alternative is to establish the mean of several analysts as the standard.

When the mean count in this study was established as the standard, analysts' counts were within 5% on 60, 67, 68, and 69% of the plates in the 10-100, 20-200, 30-300, and 40-400 colonies per plate ranges, respectively. Similarly, analysts counted within 10% of the mean count on 87, 91, 91, and 92% of the plates. Within 5 and 10% of the mean count, the count range of 10-100 was significantly different from the ranges of 20-200, 30-300 and 40-400 at the 5% level. No significant differences were detected between the 20-200, 30-300, and 40-400 count ranges.

The mean counting time to count SMA plates in the 10-100, 20-200, 30-300, and 40-400 count ranges was 18, 30, 41, and 52 seconds per plate, respectively.

CONCLUSIONS:

1. The mean of the counts of several analysts provides a measurably consistent but low estimate of the true colony count.
2. Any difference in accuracy between the colony count ranges of 20-200 and 30-300 colonies per plate is not significant at the 5% level.
3. Reducing the count range from 30-300 to 20-200 colonies per plate will reduce the time required by analysts to count plates by 27%.
4. The accuracy standards of reproducing one's own counts within 5% and the counts of others within 10% are unrealistic.

RECOMMENDATIONS:

Information obtained in this study should be utilized in the periodic review of laboratory procedures prescribed by SMEDP and AOAC. The information obtained in this study should be used in the evaluation of automated counting machines.

PUBLICATIONS:

Fruin, J.T., T.M. Hill, J.B. Clarke and J.L. Fowler. A statistical comparison of analyst accuracy and speed in counting standard methods agar plates within selected colony count ranges. Submitted to the LAIR Publications Review Committee as a LAIR Institute Report.

STUDY NO. 12 Investigations into the Microbiological Quality of Food Items Purchased by DoD for the Purpose of Establishing Microbiological Guidelines for Military Subsistence

EXP-3 A Survey of the Microflora of Fresh and Frozen Seafood with Special Emphasis on the Isolation and Identification Procedures for *Vibrio parahaemolyticus*

PROBLEM:

In recent years the subject of microbiological standards for food products has received increasing governmental attention. DoD currently has microbiological guidelines incorporated into the procurement specifications for 59 food items. These specifications do not include any fresh and frozen meat or seafood items. The enactment by state and local governments of microbiological standards for red meat items and the proposed standards and guidelines by other governmental agencies have stimulated interest in the establishment of microbial limits for other food products, including seafoods. In 1974, the International Commission on Microbiological Specifications for Foods (ICMSF) published recommended microbial limits for a number of seafood products. In addition, the U.S. Food and Drug Administration is currently analyzing frozen breaded fish and shellfish products for the purpose of establishing microbiological quality standards.

Vibrio parahaemolyticus has been identified as the cause of several food poisoning outbreaks in the U.S. with the vehicle of transmission being improperly prepared seafood. Since there is a potential hazard in the consumption of seafood products, this study was initiated to determine the microbiological quality of these food items and at the same time evaluate the adequacy of current methodology used in testing for *V. parahaemolyticus*.

RESULTS AND DISCUSSION OF RESULTS:

The microbiological quality of four frozen and seven fresh seafood products (597 units in total) obtained from a local retail store were analyzed. Aerobic plate count (APC) means (geometric) ranged from $3.5 \times 10^3/\text{g}$ to $9.3 \times 10^4/\text{g}$ for the frozen products and from $7.8 \times 10^4/\text{g}$ to $2.7 \times 10^8/\text{g}$ for fresh products. All of the frozen products tested were acceptable by the ICMSF recommended limit of $1.0 \times 10^7/\text{g}$. However, only 39.1% of the fresh seafood samples were in compliance with the ICMSF limit.

Average (geometric) coliform most probable number (MPN) values ranged from 1.0/g to 7.7/g for the frozen items and from 7.8/g to 4.8×10^3 /g for the fresh seafoods. These values correspond to the findings of other researchers for similar products.

Employing the MPN method, 4.7% of the samples analyzed were positive for *Escherichia coli*, while 7.9% were positive for *Staphylococcus aureus*. Two percent of the samples contained *Clostridium perfringens*; neither salmonellae nor *V. parahaemolyticus* were isolated from any of the units tested.

CONCLUSIONS:

The data obtained in this study indicate that the microflora of seafood products varies considerably. Although many of the products had high APCs, all products analyzed were considered acceptable for consumption.

The analysis of the eleven different seafood products did not provide sufficient information on which to base guidelines. This study did indicate that several seafood products could cause public health problems if handled improperly.

The current methodology for detecting *V. parahaemolyticus* in seafood products seems adequate. Although *V. parahaemolyticus* was not isolated in the samples tested, procedural and media controls for this organism consistently give positive results.

RECOMMENDATIONS:

Information obtained in this study has been added to the data base for these products and should be used in the event that guidelines are to be established in the future. Research and data gathering efforts should continue in this area to identify potential problem products.

PUBLICATIONS:

Foster, J.F., J.L. Fowler and J. Dacey. A microbial survey of various fresh and frozen seafood products. J. Milk Food Technol. (In press.)

EXP-5 Microflora of Ground Turkey

PROBLEM:

Comminuted turkey meat is one of several suggested alternatives for ground beef products that are often consumed in a semicooked form. Frequently, turkeys have been implicated in foodborne outbreaks of salmonellosis. Consumption of semicooked comminuted turkey meat, possibly containing *Salmonella* organisms, is a potential health hazard for military personnel and dependents. This study was undertaken to examine the product and identify the bacterial and viral flora present

as well as to increase the data base for comminuted meats on which microbiological limits for such items in the military food chain could be based.

RESULTS AND DISCUSSION OF RESULTS:

During this study fresh, frozen, and preseasoned comminuted turkey meats were examined. Analyses performed on each sample included the standard plate count, coliform plate and MPN counts, *Escherichia coli* plate and MPN counts, *Staphylococcus aureus* MPN counts, a determination of the presence of *Clostridium perfringens*, fecal streptococci MPN, as well as a selective procedure for isolation of salmonellae and the isolation and identification of aerobic, mesophilic bacterial species.

The mean standard plate count of the fresh comminuted turkey samples was 8.4×10^7 /g. The level of *E. coli* in this product, determined by the MPN method, was 19/g. *S. aureus* was isolated from 80% of the samples with a mean count of 34/g. Fecal streptococci, with a mean count of 1.8×10^4 /g, were isolated from 95% of the samples tested. *C. perfringens* and *Salmonella* sp. were isolated from 42% and 28% of the samples, respectively. The majority of *Salmonella* sp. isolated were identified as *S. enteritidis* bioserotype *pullorum* which has a low level of infectivity for man. In addition to the above, numerous bacterial species were isolated and identified. An enteric viral screen was conducted on fresh turkey samples, but no viral species were recovered.

Preseasoned comminuted turkey meat samples tested had a mean standard plate count of 2.2×10^8 /g; mean coliform plate count of 2×10^5 /g, and an *E. coli* count of 8.7/g. The low level of *E. coli* and the failure to isolate any *Salmonella* sp. from samples of this product may be a result of the addition of the spices which have been shown to influence the viability of coliform organisms. *S. aureus* was isolated from 83% of the samples with a mean level of 190/g. Fecal streptococci, with a mean count of 3.5×10^4 /g, were isolated from all samples. Fifty percent of the samples yielded isolates of *C. perfringens*. No viral studies were conducted on the preseasoned samples.

Frozen comminuted turkey meat samples had the following mean levels/g for the analyses performed: standard plate count, 9.4×10^7 ; psychrotrophic plate count, 2.4×10^6 ; coliform plate and MPN counts, 47 and 110, respectively; *S. aureus* MPN, 6; and fecal streptococci, 180. *C. perfringens*, known to be sensitive to freezing, was isolated from 20 percent of the samples. *Salmonella* sp. were isolated from 38% of samples analyzed. All but one of these *Salmonella* isolates were serotypes readily capable of causing foodborne infection. Five of the samples tested contained multiple *Salmonella* serotypes.

CONCLUSIONS:

It was concluded from this study that the comminuted turkey product, whether fresh, frozen, or preseasoned, carried a dense and diverse microbial load as well as the ability to convey salmonellae and other enteric bacilli, staphylococci and clostridia to the surfaces of equipment and the hands of kitchen workers.

RECOMMENDATIONS:

This product should not be used as an alternative to ground beef in meals without thorough cooking.

PUBLICATIONS:

Guthertz, L.S., J.T. Fruin, D. Spicer and J.L. Fowler. Microbiology of fresh comminuted turkey meat. J. Milk Food Technol. 1976. (In press).

Guthertz, L.S., J.T. Fruin and J.L. Fowler. Microbial flora of pre-seasoned comminuted turkey meat. A research note. J. Food Protection 1976. (In press).

Guthertz, L.S., J.T. Fruin, R.L. Okoluk and J.L. Fowler. Microbial quality of frozen comminuted turkey meat. Manuscript submitted to LAIR Publications Review Committee for publication.

EXP-6 A Survey of the Microbial Flora of Soya Protein Extended Ground Beef and Its Components

PROBLEM:

The United States Armed Forces consumes an estimated 50,000,000 pounds of ground beef annually and it has been estimated that the annual consumption of ground beef items will increase in the future. The cost of red meat items have been increasing steadily and budget cuts within DoD have become more frequent. In order to cope with these problems, the military services must investigate the use of other less expensive but equally nutritious food sources. The use of soya protein extended ground beef has been proposed as a partial solution to this problem. Nutritionally, this product is similar to regular ground beef. Economically, it has been shown that 20% soya extended ground beef can reduce ground beef costs by 21% on a raw basis and as much as 30% on a cooked basis. Ground beef patties containing 20% soy protein concentrate are about equal in flavor, appearance, aroma, juiciness and overall acceptability compared with all beef patties.

Although the physical characteristics of soya-extended ground beef have been investigated, the question of wholesomeness from a bacterial standpoint has not been addressed. Microbiological data on ground beef

with added soy protein is almost nonexistent. If this product is to be used by the military services an increased understanding of the bacterial flora is in order. This study was initiated to quantitate and characterize the bacterial flora of this product initially and after seven days storage at 4 C. The resulting data will be used to determine microbiological recommendations for soya protein extended ground beef.

RESULTS AND DISCUSSION OF RESULTS:

At this time, 25 lots of the product have been analyzed. Investigations will continue until a minimum of 30 lots of the product have been analyzed.

RECOMMENDATIONS, CONCLUSIONS AND PUBLICATIONS:

None; the study has not been completed.

STUDY NO. 13: Investigations into the Use of Group D Streptococci as Indicators of the Hygienic Quality of Frozen Food Products

PROBLEM:

Current microbiological methodology for the examination and determination of the hygienic quality of frozen food products is partially based upon detection and enumeration of *Escherichia coli*. Several reports have indicated that the numbers of *E. coli* which can be recovered from food products after frozen storage decrease as the length of the frozen storage period increases. If indicator organisms are to be used in determination of the hygienic quality of a frozen food item, the organisms must be able to survive freezing, and subsequent frozen storage. In addition, indicator organisms must be recoverable on the selective type bacteriologic media used in these types of analyses.

This study was undertaken to investigate the use of Group D streptococci as indicators of the hygienic quality of frozen food products.

RESULTS AND DISCUSSION OF RESULTS:

In addition to *E. coli*, all species belonging to the Group D streptococci have now been examined. Harvested cell crops of each organism were stored at -4 C, -20 C, and -80 C in the following menstrua: water, 0.2 μ phosphate buffer pH 7.0, 10% sucrose, 5% glycerol, and a 15% slurry of mashed potatoes. At preselected intervals, cell suspensions were thawed and viable cell determinations were made on selective and non-selective media.

It appears that the enterococcal portion of the Group D streptococci

are all able to withstand the effects of the freezing process and long term frozen storage at all of the temperatures and in all of the menstrua used in this study. Following thawing, these organisms were all readily recoverable on selective media.

The non-enterococcal portion of the Group D streptococci includes *Streptococcus bovis* and *Streptococcus equinus*. Data generated from these experiments indicate that during frozen storage of these organisms there is a gradual decrease in cell populations and that this decrease proceeds most rapidly at -4 C. It appears that 10% sucrose enhances the ability of *S. bovis* to survive freezing at -20 C and -80 C, however, there is impairment of the ability to recover the organism on selective media. The slurry of mashed potatoes seemed to provide some protection against the effects of frozen storage on *S. equinus* at each of the temperatures used while none of the other menstrua used could provide such protection at all temperatures.

Results of the study with *E. coli* indicate that this organism is fully capable of surviving frozen storage at each temperature in each menstrua used. There is, however, impairment of the ability to recover this organism on selective media, even after only 1 day of frozen storage.

CONCLUSIONS:

The inability to recover *E. coli* on selective media following frozen storage makes this organism unsatisfactory as an indicator organism in frozen food products. The enterococcal portion of the Group D streptococci contains several species whose potential as indicator organisms in frozen products should be given further consideration.

RECOMMENDATIONS:

It is recommended that rapid enumeration and speciation methodology be studied for the enterococcal portion of Group D streptococci and that microbial guidelines be developed for frozen food items using the enterococci as indicator organisms.

PUBLICATIONS:

None

STUDY NO. 14 Typing *Clostridium perfringens* Isolated from Foods

PROBLEM:

Clostridium perfringens type A has been recognized as a major cause of foodborne illness in the United States since the 1960s. In 1973 the organism was confirmed as the causative agent for 1,424 cases of foodborne illness in the U.S. The organism can routinely be isolated

from food, soil, air, water, and the intestinal contents of man and animals.

Based on exotoxins produced in culture filtrates, five types (A through E) of *C. perfringens* have been identified. All types are pathogenic to man, animals or both, but only type A plays a significant roles in foodborne illnesses in the U.S.

Standard laboratory procedures for the microbiological analysis of food samples identify the organism as *C. perfringens* but do not attempt to determine the type. Since types B, C, D, and E are not associated with foodborne illness, their presence in food samples is presumably of limited public health significance. Thus, if a high percentage of *C. perfringens* isolated from food items are of a type other than type A, additional laboratory procedures are necessary to determine if a food has the potential to cause foodborne illness.

RESULTS AND DISCUSSION OF RESULTS:

Over one hundred and fifty strains of *C. perfringens* isolated from food samples have been evaluated by using the mouse toxin neutralization test. The predominant type isolated has been A. Definitive typing of strains other than type A will be completed when the collection of new strains has been terminated.

CONCLUSIONS, RECOMMENDATIONS, AND PUBLICATIONS:

None; this study has not been completed.

STUDY NO. 15 The Effect of Cryoprotective Agents in the Survival of
Clostridium perfringens Type A Vegetative Cells in
Selected Meat Products After Freezing and Thawing

PROBLEM:

Until 1953, *C. perfringens* was not recognized as a foodborne illness entity. During the past five years, this organism has been responsible for over 25% of the confirmed cases of foodborne illness in the U.S. Historically, the causative agent of foodborne illness is confirmed in about 50% of the reported cases. Confirmation probably is even lower in nonreported outbreaks. It is probable that *C. perfringens* is involved in many of the nonreported outbreaks. The organism is an anaerobe, and is susceptible to destruction at low temperatures, particularly freezing. As a result, the collection, handling, shipment, storage and laboratory preparation of samples often has a detrimental effect on the recovery of *C. perfringens*. Cryoprotective agents, and agents that reduce the oxidation reduction potential of food samples, could enable laboratories to detect the presence of *C. perfringens* with more precision.

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RESULTS AND DISCUSSION OF RESULTS:

Freezing active cultures of *C. perfringens* vegetative cells in a laboratory preparation of cooked meat with various chemicals added has been initiated. Ethylene glycol, methanol, dimethyl foramide, propylene glycol, and glycerol added to the cooked meat medium provided the vegetative cell more protection than cooked meat alone. Several sugars, urea, phenol, and sodium chloride had a detrimental effect on survival of *C. perfringens* cells. Several other chemical compounds remain to be screened.

CONCLUSIONS:

Preliminary indications suggest that some chemicals are at least partially effective as cryoprotective agents.

RECOMMENDATIONS AND PUBLICATIONS:

None; this study has not been completed.

STUDY NO. 16 Computerized Data Collection Program in Food Microbiology

PROBLEM:

Regulatory food microbiology is an area which requires a sound data base on which to base standards, guidelines, or specifications scientifically. Since the adoption of standards requires the passage of an implementing law, DoD has used military specifications as the vehicle for imposing microbiological criteria on food items purchased for military consumption. It is unfortunate that some of these criteria were developed with an inadequate data base. They need to be re-examined now that up-to-date microbiological data are available.

A Computerized Food Microbiological Data Collection Program was designed and implemented under another work unit in 1972. The data base now contains in excess of 40,000 entries of food microbiological test results, and provides a firm basis for formulating guidelines or military specifications on a number of products. Additionally, military public health information is readily available from the file.

RESULTS AND DISCUSSION OF RESULTS:

Basic statistics for the years 1972-1975 are tabulated in Table 1.

TABLE 1

	<u>1972</u>	<u>1973</u>	<u>1974</u>	<u>1975</u>
Number of Participating Laboratories	11	11	10	10
Total Reports	6,120	7,639	7,409	7,346
Total Messages in File	26,361	31,426	31,190	26,461
Standard Plate Counts Performed	5,398	6,037	6,121	6,041
Coliform Counts Performed	5,381	5,564	5,380	5,272
Yeast and Mold Counts Performed	2,444	2,578	2,395	1,760
<i>E. coli</i> Counts Performed	2,436	1,604	3,366	3,709
Salmonella Analyses Performed	1,451	1,546	1,924	1,838
<i>C. perfringens</i> Counts Performed	622	770	600	1,041
Other Clostridia	134	194	106	37
Types of Foods Analyzed	793	810	831	884

The computer program has proven to be extremely flexible and adaptable. The internal editing system, which precludes transfer of data to the master file unless verified by a cross-check dictionary file, has considerably reduced the cost of verifying the data base. Slight modification of the program has made it possible for computer-created graphs of microbiological data within the file to be generated. Military public health data, procurement data, and identification of problem products can easily be retrieved by proper use of the file.

RECOMMENDATIONS AND CONCLUSIONS:

With the addition of data to the file on an annual basis, it is now possible to formulate tentative guidelines on several food items. Products previously identified in this category are comminuted beef products, prepared sandwiches, and delicatessen salads. Specific microbiological guidelines for these products, based on data from this program, are:

1. A standard plate count limit of 10^7 /g appears to be feasible

and reasonable for ground beef. A total coliform limit of 10^4 /g also appears feasible.

2. A standard plate count limit of 10^5 /g as well as a total coliform count limit of 100/g appear to be feasible as microbiological criteria for prepared sandwiches sampled at military facilities.

3. A standard plate count limit of 10^5 /g and a total coliform limit of 100/g appear to be feasible as microbiological criteria for delicatessen salads.

It is recommended that this program be continued, and that maximum use be made of information in the file to formulate food microbiological guidelines. Data should be made available to interested civilian sectors.

PUBLICATIONS:

Fowler, J.L., D.L. Stutzman, J.F. Foster and W.H. Langley, Jr. Report of 1973 Microbiological Data Collection Program. LAIR Rpt 27. 1975.

Fowler, J.L., D.L. Stutzman, J.F. Foster, W.H. Langley, Jr., and K.E. Trefz. Report of 1974 Microbiological Data Collection Program. LAIR Rpt 28. June 1976.

Fowler, J.L., D.L. Stutzman, J.F. Foster, W.H. Langley, Jr., and K.E. Trefz. Report of 1975 Microbiological Data Collection Program. LAIR Rpt. (In press).

Fowler, J.L. Microbiological data of comminuted meat products from military sources - A 3-year compilation. Minutes of Joint Meeting of the National Association of State Meat and Food Inspection Directors and American Association of Veterinary Food Hygienists. Portland, OR. November 1975.

Fowler, J.L. Microbiological data of comminuted meat products from military sources - A 3-year compilation. Abstracted in Food Chemical News. 15 December 1975.

Fowler, J.L., D.L. Stutzman, J.F. Foster and W.H. Langley, Jr. Selected food microbiological data collected through a computerized program. J. Milk Food Technol. 1976. (In press).

STUDY NO. 17 Investigations into Potential Toxicological Problems Associated with Food Items Purchased by DoD: Identification of Hazardous Substances, Evaluation of Food Safety, Alleviation of Toxicological Problems and Establishment of Tentative Toxicological Guidelines for Military Subsistence

EXP-1 Histamine Production by Food Spoilage Microorganisms
and Development of an Analytical Method for Detection
of Histamine in Food Products

PROBLEM:

Histamine in foods, particularly in certain seafood products and various fermented foods, has been implicated as the causative agent in several recent foodborne disease outbreaks. A need exists to develop a sensitive and specific analytical method for detection of histamine for use with a wide variety of food products. Various selective extraction procedures coupled to several different fluorometric and spectrophotometric detection methods will be explored. Various chromatographic procedures including thin-layer chromatography, column chromatography, gas-liquid chromatography, and high pressure liquid chromatography will be evaluated for their potential in removal of any remaining interfering substances.

Various species of microorganisms serve as the major sources of histamine formation in foods. A number of microbial species possess the required enzyme, histidine decarboxylase, and can therefore produce histamine as a decarboxylation product of histidine. However, a need exists to determine quantitatively the relative histamine production by common species of food spoilage microorganisms on chemically defined and ground fish media. Once the key microbial species in terms of quantitative histamine production are identified, the optimal conditions for their growth and histamine production in food products will be assessed.

RESULTS AND DISCUSSION OF RESULTS, CONCLUSIONS, RECOMMENDATIONS, AND PUBLICATIONS:

None; this study has not been completed.

EXP-2 The Toxicity of Histamine and Other Biogenic Amines as
Related to Food Poisoning, the Presence of Inhibitors
of Histamine-Metabolizing Enzymes in Foods, Dietary
Conditions, and Other Gastrointestinal Factors

PROBLEM:

Histamine and other biogenic amines, particularly tyramine, have been implicated in numerous foodborne disease outbreaks. The correlation between histamine levels and disease occurrence is well established. However, histamine administered orally in large quantities to laboratory animals causes no apparent ill-effects. The gastrointestinal tract normally contains three enzymes - monoamine oxidase, diamine oxidase, and histamine-N-methyltransferase - which are capable of metabolizing and thereby detoxifying histamine. With tyramine the disease symptoms usually occur only after ingestion of a tyramine-

containing food along with a monoamine oxidase inhibitor such as tranylcypromine. With cases of food poisoning due to histamine, particularly scombroid fish poisoning, such obvious mitigating factors have not been uncovered. The possible existence of naturally-occurring or additive foodborne inhibitors of histamine metabolizing enzymes needs to be investigated as a possible solution to this paradox. A number of potential inhibitors will be tested against the three enzymes which will be partially purified from rat small intestine.

In addition, diamine oxidase may be sensitive to pyridoxal and riboflavin in the diet while histamine-N-methyltransferase may require adequate nutritional supplies of folate and vitamin B₁₂. The effect of these factors on histamine detoxification by intestinal enzymes will also be investigated. The effects of these dietary factors will be assessed on the basis of in vitro enzyme activity determinations and ligated gut experiments on ¹⁴C-histamine absorption. The in vivo effect of these factors will be studied after intraduodenal injection of histamine by observing changes in stomach acid secretion volume and acidity.

Other gastrointestinal factors, particularly microorganisms with histaminase activity, will be investigated for their potential effect on histamine toxicity also.

RESULTS AND DISCUSSION OF RESULTS, CONCLUSIONS, RECOMMENDATIONS, AND PUBLICATIONS:

None; this study has not been completed.

STUDY NO. 18 Investigations into Methodology for Detecting and Identifying Viruses in Selected Department of Defense Subsistence Items

PROBLEM:

The role of food as a vehicle for virus transmission to humans has not been clearly defined. In the latest annual report of the Center for Disease Control, 3% of the confirmed outbreaks of foodborne disease were of viral etiology. However, an etiology was confirmed in only 44% of the outbreaks. It is possible that viruses contribute a significant part of the outbreaks of unknown etiology since laboratory methodology for detection and identification of viruses in foods are not well established. Most of the reports of foodborne viral disease outbreaks are based on epidemiological evidence only and are limited to those diseases with a distinct clinical syndrome, i.e., hepatitis and paralytic poliomyelitis. There have been only a few reports of recovery of viruses found in food items except those found in seafoods. Now, there are a few preliminary reports on the methodology for detecting enteroviruses in foods. These studies involve the laboratory contamination of food items and efforts to recover virus.

The purpose of this study is to establish methods for isolation and identification of virus from food items and to perform viral surveys on a limited number of foods of military importance.

RESULTS AND DISCUSSION OF RESULTS:

Work thus far has involved the establishment of cell cultures, positive control virus stocks, viral plaque techniques and the evaluation of procedures for the recovery of virus from ground beef. The efficiency of virus recovery is determined by adding a known quantity of virus to a ground beef sample and then measuring the quantity of virus present following processing. Upon completion of the evaluation of sample processing procedures, selected food items (ground beef, pork sausage, etc.) will be tested for the presence of virus.

RECOMMENDATIONS, CONCLUSIONS, AND PUBLICATIONS:

None; this study has not been completed.

STUDY NO. 19 Investigations into Potential Insect Induced Toxicological Problems in Food

EXP-2 Benzoquinones from the Secretory Glands of *Tribolium confusum* and *Tribolium castaneum* - Assay, Reactions, Effects, and Toxicity

PROBLEM:

Insect damage to DoD subsistence items is appreciable and costly. The criteria for disposal of infested subsistence materials is based on crude measurements such as whole insect or fragment counts. From the standpoint of military public health, criteria based on the level of toxic insect products would be more reliable and justifiable. However, little information exists on toxic chemicals produced by insects. It is known that many surface feeding insects including the common flour beetles, *Tribolium confusum* and *Tribolium castaneum*, produce benzoquinones from secretory glands. These benzoquinones have some reported toxicity although the toxicity evaluations were based on poorly designed experiments. A need exists to determine the acute and chronic toxicity of the benzoquinones. The reactions of these benzoquinones with food components and the effect of such reactions on food quality and toxicity needs to be evaluated. The reactions of the benzoquinones with various biomolecules also require investigations, and may provide clues toward the biochemical basis of toxicity. The analysis of these compounds in food products is necessary to development of any toxicological guidelines and therefore, a variety of potential analytical techniques will be evaluated.

RESULTS AND DISCUSSION OF RESULTS, RECOMMENDATIONS, CONCLUSIONS, AND PUBLICATIONS:

None; this study has not been completed.

ADDITIONAL PUBLICATIONS UNDER WORK UNIT 083:

STUDY NO. 11 Fowler, J.L., W.S. Clark, Jr., R.T. Sterner, and J.F. Foster. Analyst variation in doing the standard plate count as described in "Standard Methods for the Examination of Dairy Products." Submitted for publication.

Fruin, J.T. The fate of *Clostridium perfringens* during the processing of foods. (Abstract) J. Am. Vet. Med. Assoc. 1976. (In press)

Fruin, J.T. The significance of *Clostridium perfringens* in processed foods. Submitted for publication.

RESEARCH WORK PERFORMED ELSEWHERE BUT PUBLISHED DURING FY 76:

1. Nowicki, H.G., E.R. Lieber and R.E. Dolle. Applications of gas chromatography-mass spectrometry to analysis of crude opium, codeine, dihydrocodeine, and diacetylmorphine. Abstracts of Papers, American Chemical Society 172nd National Meeting, San Francisco, CA. 1976. Paper #100, Analytical Chemistry.
2. Nowicki, H.G., E.R. Lieber, R.E. Dolle, R.P. Erickson, R. Wojciechowski, J.H. Stopper, II and M.D. Milton. Impact of gas chromatography-mass spectrometry on forensic-toxicological chemistry. Abstracts of Papers, American Chemical Society 172nd National Meeting, San Francisco, CA. 1976. Paper #101, Analytical Chemistry.
3. Taylor, S.L. and A.L. Tappel. Effect of dietary antioxidants and phenobarbital pretreatment on microsomal lipid peroxidation and activation by carbon tetrachloride. Life Sci. 1976.
4. Taylor, S.L., M.P. Lamden and A.L. Tappel. Sensitive fluorometric method for tissue tocopherol analysis. Lipids. 11: 530. 1976.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTRN ^a	8B. SPECIFIC DATA CONTRACTOR ACCESS ^a	9. LEVEL OF SUM ^a
75 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A811		00		001	
b. XXXXXXXXXX	672760A	3A762760A822		02		086	
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(U) Nutrition Studies in Support of DOD Food Program							
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13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 07		CONT		DA		C. In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
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19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME ^a Letterman Army Institute of Research Department of Nutrition, Information Sciences & Administration Presidio of San Francisco, CA 94129			
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Foreign Intelligence Not Applicable				NAME: Chastain, R., CPT, MS, Nelson, R.A.			
				NAME: Skala, J.H., Johnson, H.L., DAC POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Rations; (U) Nutritional Requirements; (U) Military Medicine; (U) Clinical Chemistry; (U) Analytical Biochemistry; (U) Diet							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To provide capabilities to respond to the needs and requirements of the DOD Food RDT&E program (AR 70-3). Capabilities are provided to organize and participate in military nutrition surveys. These surveys as requested are performed for all of the military services and are conducted in CONUS and OCONUS. Objectives are: (a) to provide essential information and guidance pertaining to the nutritional adequacy of the feeding systems, rations and dietary standards employed by the military services in various environs; (b) to evaluate the nutritional status, nutrient intake, physical status, body composition and work capacity of military personnel to ensure that performance is not impaired by improper nutrition; (c) to evaluate military rations existent and experimental in terms of chemical composition and nutrient content; and (d) to provide statistical and computer support for the indicated objectives.</p> <p>24. (U) Information is obtained through procedures such as clinical examinations and histories, dietary recalls, food intake measurements, nutrient analyses of food items and composites, biochemical measurements on blood and urine samples, body composition measurements and physical performance tests. Data will be subjected to mathematical and statistical evaluations through the use of developed computer programs, data files and data reduction systems. Specific and unique military nutrition and dietary problems posed by any of the Services would receive special attention through the conduct of workshops, symposia or research.</p> <p>25. (U) 75 07 - 76 09 The 2nd phase of the study of the Loring AFB a la carte system was completed. Data were collected for 5 days on foods served, plate wastes, food selections and participation rates. A laboratory report is being finalized. Final phases of the NAS Alameda survey have been completed. An interim report has been drafted on dietary intake information obtained by interview techniques.</p>							

DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A762760A822	Military Internal Medicine
TASK NO.	02	Nutrition and Wholesomeness Support for DoD Food Program
WORK UNIT NO.	086	Nutrition Studies in Support of DoD Food Program

The following investigations have been conducted under this work unit:

STUDY NO. 1 Nutrition Surveys of Military Populations and
Installations:

- b. Loring Air Force Base (AFB), Maine, Nutrition
Survey, 1974-1976
- f. Naval Air Station (NAS), Alameda, California,
Nutrition Survey, 1975-1976

Study No. 1-b. A study of the impact of changing to an all basic allowance for subsistence (BAS)/a la carte item priced feeding system upon dining hall attendance, plate wastes, and nutrient consumptions was conducted at Loring AFB by two short dining hall surveys: Oct 1974 upon the "old" system and Nov 1975 upon the BAS/a la carte feeding system. Providing all of the men with their basic allowance for subsistence and operating the dining hall like a cafeteria reduced attendance 19%, reduced plate wastes from 9.35 to 5.5% of the total food served, and altered the consumption pattern among the various food groups. Military dining hall meals do not meet the goal of a maximum of 40% of the calories from fat either under the "old" system or under the BAS/a la carte system.

Study No. 1-f. The final phases of the nutrition survey of the NAS, Alameda, CA, were completed. The objective of the survey was to evaluate the impact of conversion from the existing mixed ration-in-kind (RIK)/commuted ration (COMRAT) standard dining hall to an all-COMRAT/cash a la carte system on nutrient intake and nutritional status of enlisted personnel. Conversion of personnel to a commuted ration status appears to have had an adverse effect upon their nutritional status. An interim report is being completed.

BODY OF REPORT

WORK UNIT NO. 086 Nutrition Studies in Support of
DoD Food Program

TASK NO. 02 Nutrition and Wholesomeness
Support for DoD Food Program

STUDY NO. 1 Nutrition Surveys of Military Populations and
Installations

b. Loring Air Force Base (AFB), Maine, Nutrition Survey, 1974-1976

PROBLEM:

The US Air Force has been promoting the basic allowance for subsistence (BAS)/cash a la carte feeding system and has conducted two tests of this system, one at Shaw AFB in 1973 and another at Loring AFB in 1975. Letterman Army Institute of Research (LAIR) was requested to obtain information on the impact of the new feeding system at Loring AFB. Data were to reflect the nutrient consumption of the personnel and the amount of plate waste. Therefore, two limited nutrition surveys were conducted on the military dining hall at Loring AFB. The baseline or control data were obtained from a 3-day survey of the "old" feeding system in Oct 1974 and the BAS/cash a la carte system was surveyed during Nov 1975.

RESULTS AND DISCUSSION OF RESULTS:

The statistical evaluations and drafting of the LAIR Report with analyses of the nutritional impact and effect upon food wastes are presently ongoing. Some of the preliminary findings are presented in Tables 1 through 6. Plate waste comparisons by food group, Table 1, shows reductions for the BAS/a la carte systems for all groups except citrus fruits; the total wastes, based on total grams served, were reduced from 9.35 to 5.25%. Comparing the BAS/a la carte system to the Oct 1974 systems shows: (a) the amount of meat, fish and poultry served was reduced while consumption was not changed, (b) decreases in the consumption of egg and egg products (43%), leafy green and yellow vegetables (14%), tomatoes (16%), citrus fruits (66%), other vegetables (14%), other fruits (54%), and desserts (65%), (c) increases in the consumption of grain products (48%), white potatoes (9%), carbonated and non-carbonated beverages (84%), and soups (77%), and (d) no significant changes in the other consumptions.

The average headcounts and average nutrient consumptions per man per meal for each meal of the day are shown in Tables 2 to 6. The total number of meals served in this dining hall decreased 19% during the Nov 1975 survey (BAS/a la carte) compared to Oct 1974 with decreases at all meals except the midnight meal (29% increase). Although the

short order noon meal increased 117% in attendance, the main meal decreased 45% at that time with a net 17% decrease in number of personnel served. With the a la carte system, 45% of the dinners were short order compared to only 17% under the previous feeding system. Caloric consumptions for breakfast meals were not changed after changing to the a la carte system although protein intakes increased 9%, while fat and carbohydrate decreased 17% each. The dinner (noon) meals consumed under the new system contained less nutrients with the greatest reductions from the short order meals. Calories were reduced 24 and 34% (main line and short order, respectively), protein, 6 and 37%; fat, 37 and 49%; and carbohydrate, 14 and 24%. In the 1975 supper meals, an average of 9% more calories, 17% more protein, 10% more fat, and 3% more carbohydrates were consumed, than were consumed in 1974. All macro nutrient consumptions were reduced at the midnight meal for the BAS/a la carte system, calories (9%), protein (6%), fat (2%) and carbohydrate (19%). Percent protein calories increased in 1975 for all meals except short order dinners (means ranging from 15 to 19%). The contribution of fat to the caloric intakes was too high and increased in 1975 for breakfasts from 41 to 47%, and midnight meals from 45 to 49%; however, decreases were noted for main dinners (49 to 40%) and short order dinners (44 to 40%); suppers were unchanged. Vitamin and mineral intakes were generally adequate in both surveys for all meals with the possible exception of niacin at breakfasts.

CONCLUSIONS AND RECOMMENDATIONS:

1. BAS/a la carte system reduced plate wastes.
2. There was a decrease of 19% in the number of meals served per day in comparing the 1975 BAS/a la carte system to the 1974 "old" system of feeding.
3. The military dining halls serve meals containing too much fat, 40 to 49% of the calories.
4. Milk consumption is reduced as soft drink consumption increases; however, calcium intake was adequate.
5. Consumptions of fruits and vegetables were greatly reduced after changing to the BAS/a la carte system and this should be reversed (by subsidizing these items if necessary).

STUDY NO. 1 Nutrition Surveys of Military Populations and
Installations

f. Naval Air Station (NAS), Alameda, CA 1975-1976

PROBLEM:

A nutrition survey was conducted at Naval Air Station, Alameda, California, to evaluate the impact of conversion from the existing mixed ration-in-kind (RIK) commuted ration (COMRAT) standard dining hall system, to an all-COMRAT/cash a la carte system on nutrient intake and nutritional status of enlisted personnel. Phase I was conducted in March 1975, to study the existing mixed RIK/COMRAT system. Phase II was conducted in June 1976, as an interim study following conversion to the cash a la carte system which was implemented on 1 March 1976. Phase III was conducted in August, 1976, following a 5-month stabilization period. Phases I and III encompassed dining hall, biochemical/clinical examination, and total dietary intake aspects. Only the total dietary aspect was performed in Phase II.

RESULTS AND DISCUSSION OF RESULTS:

The results reported are derived solely from data obtained by the dietary interview technique from 35 ration-in-kind (RIK), 50 commuted ration-single (COM-S) and 48 commuted ration-married (COM-M) participants in the 17-day March 1975 survey, and from 41 RIK, 50 COM-S, and 63 COM-M participants in the 14-day June 1976 survey at NAS, Alameda, CA. An interim report of these results has been drafted. Data obtained from the dining hall (e.g., amounts of foods served and consumed, plate waste, calculated and analyzed nutrient composition of foods served, and nutrient intakes per dining hall meal of the individual dining hall patron), clinical, biochemical, and demographic aspects of the study are being analyzed and evaluated.

Conversion of RIK personnel to commuted ration status markedly reduced dining hall utilization from 31.6% in March 1975 to only 11.1% in June 1976 (Table 7). The a la carte item-pricing system was not effective in stimulating a compensatory increase in dining hall utilization of COM-S (8.2% in 1975 vs. 10.7% in 1976) and COM-M (1.0% in 1975 and 5.1% in 1976) personnel. Dining hall utilization of all participants decreased from 11.8% in 1975 to 9.1% in 1976.

The average daily nutrient intake from both inside and outside dining hall sources is shown in Table 8. Conversion of RIK personnel to COMRAT status reduced their average daily intakes of food, energy, protein, carbohydrate, calcium, phosphorus, thiamin, and riboflavin, but only to levels similar to that consumed by personnel who had received commuted rations for more prolonged periods of time. Calcium: phosphorus ratios were near the upper limits of desirability.

Calories derived from fat sources were near the upper limit (40% fat calories) of desirability. Conversion of the dining hall to the cash a la carte system did not appreciably affect the total daily nutrient intake of the COM-S and COM-M groups, primarily because of their low attendance pattern at the dining hall.

Evaluation of the percentages of the various group populations having nutrient intakes below the recommended dietary allowance revealed that conversion to commuted ration status had an adverse effect upon the total nutrient intake of personnel formerly on RIK status. A greater percentage of the former RIKs in the June 1976 survey had energy, protein, calcium, thiamin, riboflavin, and ascorbic acid intakes below the recommended allowances. Analysis of nutrient intake per dining hall meal data (Table 9) indicated an adverse effect of item-pricing on calcium and riboflavin intakes almost entirely attributable to a decrease in milk and milk-product consumption.

CONCLUSIONS:

The results indicate that groups of personnel at the NAS, Alameda, who consumed a substantial number of their meals in the military dining hall had more satisfactory nutrient intakes than those groups that only infrequently patronized the dining hall. Furthermore, a significant proportion of the young, male military personnel had nutrient intakes below the recommended dietary allowances. Conversion of personnel from RIK to COMRAT status accentuated rather than alleviated the problem.

RECOMMENDATIONS:

Our recommendations based upon the nutritional impact of the cash a la carte system as tested at NAS, Alameda are as follows:

1. Discontinue conversion of all rations-in-kind personnel to commuted rations status, especially at military installations with readily available alternative food outlets.
2. If the a la carte system is continued, we strongly recommend that milk, particularly low fat or 2% vitamin A fortified milk, be item-priced sufficiently lower than cost (to be below soft drink prices) to stimulate milk consumption and thereby improve calcium, vitamin A and riboflavin intakes.
3. Further consideration should be given to modifying the item-pricing system to improve the nutritional health of military personnel.

PUBLICATIONS:

1. Consolazio, C.F. The impact of low caloric feeding during exercise. Third International Meeting on "Food for the Armed Forces", US Army Natick Research and Development Center, Natick, MA, 15-18 Oct 76. Published in Technical Report 76-42-OTD, p. 61-94.
2. Hill, T.M. Amounts and nutritional adequacy of foods consumed outside of military dining facilities. Third International Meeting on "Food for the Armed Forces", US Army Natick Research and Development Center, Natick, MA, 15-18 Oct 76. Published in Technical Report 76-42-OTD, p. 39-53.
3. Sauberlich, H.E. and J.E. Canham. Establishment and assessment of certain US military nutrient requirements. Third International Meeting on "Food for the Armed Forces", US Army Natick Research and Development Center, Natick, MA, 15-18 Oct 76. Published in Technical Report 76-42-OTD, p. 95-115.
4. Hill, T. A summary of US Army and Navy POWs of the Vietnam War. To be published in the Proc. of Joint Meeting POW/MIA Matters, Center of Prisoner of War Studies, Naval Health Research Center, San Diego, CA.
5. Schnakenberg, D.D., T.M. Hill, C.F. Consolazio and J.E. Canham. Effect of conversion to a cash a la carte feeding system on total nutrient intake of NAS/Alameda personnel. Interim Report. (Prepared for final approval).

TABLE 1
FOOD CONSUMPTION AND PERCENT PLATE WASTE
LORING AFB, MAINE

Summary by Food Type, Average, Daily

Food Item	Oct 74 - Headcount 3209			BAS/a la carte Nov 75 - Headcount 4541		
	Gm/Man	Gm/Man	%	Gm/Man	Gm/Man	%
	Served	Consumed	Waste	Served	Consumed	Waste
Meat, fish & poultry	135.8	115.3	15.1	128.5	118.1	8.1
Eggs & egg products	50.8	47.7	6.2	39.3	38.2	2.8
Milk & milk products	437.6	415.1	5.1	362.2	354.8	2.0
Butter	4.47	3.53	21.5	3.62	3.46	3.5
Fats, other	15.8	14.9	5.7	17.0	16.1	5.4
Sugars	8.67	7.87	9.4	3.54	3.38	4.7
Grain products	74.5	63.6	14.6	100.7	94.4	6.2
Legumes & nuts	16.0	12.1	24.5	13.0	11.7	10.0
Vegetables, leafy green & yellow	31.8	23.6	25.8	25.1	20.3	18.8
Tomatoes	10.1	7.87	22.7	7.35	6.58	10.3
Citrus fruits	75.1	70.5	6.1	26.3	24.2	7.7
White potatoes	61.6	55.5	9.8	65.5	60.6	7.5
Vegetables, other	14.3	12.2	14.4	11.9	10.5	11.8
Fruits, other	49.3	44.9	9.0	21.8	20.5	6.3
Beverages	46.8	42.8	8.5	81.6	78.9	3.3
Miscellaneous	2.33	2.13	6.5	1.96	1.85	5.9
Desserts	45.3	40.2	11.1	16.2	14.1	12.7
Soups	8.60	7.33	14.9	14.5	13.0	10.4

TABLE 2

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TABLE 3

1975 - Mean

TABLE 4
LORING AFB NUTRITION STUDY - SHORT ORDER DINNER

	1974 - Mean		1975 - Mean	
Number of Days	3		5	
Headcount	84		182	
Consumption	1139 Gm		887 Gm	
Water	800.2 Gm		656.4 Gm	
Energy	1718 Kcal		1129 Kcal	
Protein	76.0 Gm	(17.6% of Calories)	48.1 Gm	(16.9% of Calories)
Fat	84.0 Gm	(43.8% of Calories)	50.5 Gm	(39.9% of Calories)
Carbohydrate	166.3 Gm	(38.6% of Calories)	122.5 Gm	(43.1% of Calories)
Fiber	1.68 Gm		1.64 Gm	
Ash	12.7 Gm		8.3 Gm	
Calcium	948 Mg		460 Mg	
Phosphorus	1232 Mg	0.77 Ca/P ratio	697 Mg	0.66 Ca/P ratio
Iron	9.6 Mg		6.8 Mg	
Sodium	2104 Mg		1422 Mg	
Potassium	1959 Mg		1329 Mg	
Vitamin A	1654 IU	0.96 IU/Kcal	1845 IU	1.63 IU/Kcal
Thiamin	0.76 Mg	0.44 Mg/1000 Kcal	0.52 Mg	0.46 Mg/1000 Kcal
Riboflavin	1.65 Mg	0.96 Mg/1000 Kcal	0.92 Mg	0.82 Mg/1000 Kcal
Niacin	12.2 Mg	7.08 Mg/1000 Kcal	9.4 Mg	8.36 Mg/1000 Kcal
Ascorbic acid	57 Mg	0.16 Mg/Gm Protein		0.20 Mg/Gm Protein
		33.13 Mg/1000 Kcal	28 Mg	24.81 Mg/1000 Kcal

TABLE 5

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LORING AFB NUTRITION STUDY - MIDNIGHT

<u>Nutrient</u>	<u>1974 - Mean</u>	<u>1975 - Mean</u>
Number of Days	3	5
Headcount	56	72
Consumption	1021 Gm	894 Gm
Water	748.5 Gm	652.9 Gm
Energy	1383 Kcal	1259 Kcal
Protein	56.6 Gm	53.4 Gm
Fat	69.1 Gm	68.0 Gm
Carbohydrate	133.5 Gm	108.7 Gm
Fiber	0.87 Gm	0.95 Gm
Ash	11.2 Gm	11.0 Gm
Calcium	738 Mg	794 Mg
Phosphorus	998 Mg	998 Mg
Iron	7.4 Mg	6.8 Mg
Sodium	1771 Mg	1905 Mg
Potassium	1744 Mg	1459 Mg
Vitamin A	2919 IU	2521 IU
Thiamin	0.87 Mg	0.80 Mg
Riboflavin	1.41 Mg	1.48 Mg
Niacin	6.9 Mg	5.9 Mg
Ascorbic acid	103 Mg	33 Mg
		(16.4% of Calories)
		(45.0% of Calories)
		(38.6% of Calories)
		(16.9% of Calories)
		(48.6% of Calories)
		(34.5% of Calories)
		0.80 Ca/P ratio
		0.74 Ca/P ratio
		2.11 IU/Kcal
		0.63 Mg/1000 Kcal
		1.02 Mg/1000 Kcal
		4.96 Mg/1000 Kcal
		0.12 Mg/Gm Protein
		74.12 Mg/1000 Kcal
		2.00 IU/Kcal
		0.63 Mg/1000 Kcal
		1.18 Mg/1000 Kcal
		4.65 Mg/1000 Kcal
		0.11 Mg/Gm Protein
		25.82 Mg/1000 Kcal

TABLE 7

EFFECT OF CASH A LA CARTE SYSTEM ON DINING HALL ATTENDANCE
NAVAL AIR STATION, ALAMEDA, CA

Group	BEFORE CASH A LA CARTE (MAR 75) ¹		AFTER CASH A LA CARTE (JUN 76) ²	
	Dining Hall Meals Per Study	Percent Utilization	Dining Hall Meals Per Study	Percent Utilization
RIK	16.1 ± 1.83 ³	31.6 (35) ⁴	5.0 ± 1.14	11.1 (41)
COM-S	4.2 ± 1.00	8.2 (50)	4.8 ± 0.93	10.7 (50)
COM-M	0.5 ± 0.23	1.0 (48)	2.3 ± 0.53	5.1 (63)
ALL	6.0 ± 0.82	11.8 (133)	3.8 ± 0.49	9.1 (154)

¹ Maximum of 51 meals per 17-day study

² Maximum of 42 meals per 14-day study

³ Mean ± SEM

⁴ Values in parentheses indicate numbers of subjects completing study

TABLE 8
AVERAGE DAILY TOTAL NUTRIENT INTAKE
NAVAL AIR STATION, ALAMEDA, CA

BEFORE CASH A LA CARTE (MARCH 1975)

Nutrient	RIK	COM-S	COM-M
Quantity (gm)	2804 ± 342 ¹	2697 ± 137	2527 ± 149
Energy (Kcal)	2945 ± 200	2693 ± 141	2391 ± 78
Protein (gm)	106.1 ± 5.3	99.3 ± 4.8	95.4 ± 4.1
Fat (gm)	119 ± 5.4	114 ± 6.5	106 ± 4.1
(% Fat Calories)	38.2 ± 1.25	38.3 ± 1.03	40.0 ± 0.94
Carbohydrate (gm)	299 ± 18	262 ± 13	238 ± 10
Fiber (gm)	3.44 ± 0.31	3.48 ± 0.25	3.51 ± 0.23
Ash (gm)	17.7 ± 1.00	16.7 ± 0.82	15.9 ± 0.63
Calcium (mg)	1138 ± 80	996 ± 78	827 ± 43
Phosphorus (mg)	1594 ± 89	1452 ± 86	1357 ± 53
(CA:P)	1:1.50 ± 0.07	1:1.59 ± 0.06	1:1.74 ± 0.08
Iron (mg)	15.2 ± 0.78	16.0 ± 0.84	16.3 ± 0.97
Sodium (mg)	3220 ± 177	2943 ± 159	2765 ± 130
Potassium (mg)	3124 ± 189	2820 ± 142	2738 ± 105
Vitamin A (IU)	4540 ± 526	4968 ± 452	5414 ± 584
Thiamin (mg)	1.34 ± 0.07	1.31 ± 0.08	1.28 ± 0.06
(mg/1000 Kcal)	0.47 ± 0.02	0.48 ± 0.02	0.53 ± 0.02
Riboflavin (mg)	2.57 ± 0.15	2.26 ± 0.13	2.00 ± 0.09
(mg/1000 Kcal)	0.89 ± 0.03	0.84 ± 0.02	0.84 ± 0.03
Niacin (mg)	21.0 ± 1.22	20.6 ± 0.87	21.1 ± 1.04
(mg/1000 Kcal)	7.29 ± 0.21	7.97 ± 0.27	8.96 ± 0.36
Ascorbic Acid (mg)	62.2 ± 5.7	73.4 ± 7.3	74.8 ± 6.7

Page 1 of 2 pages

TABLE 8 (Cont)

AFTER CASH A LA CARTE (JUNE 1976)

Nutrient	RIK	COM-S	COM-M
Quantity (gm)	2247 ± 138	2388 ± 125	2672 ± 151
Energy (Kcal)	2375 ± 96	2449 ± 107	2540 ± 102
Protein (gm)	93.5 ± 3.8	96.5 ± 4.3	101.6 ± 4.4
Fat (gm)	107 ± 4.8	109 ± 5.3	114 ± 5.5
(% Fat Calories)	40.8 ± 0.84	40.2 ± 0.77	40.4 ± 0.77
Carbohydrate (gm)	235 ± 12	231 ± 10	250 ± 10
Fiber (gm)	2.94 ± 0.22	2.78 ± 0.13	3.28 ± 0.22
Ash (gm)	14.7 ± 0.71	15.5 ± 0.76	16.2 ± 0.74
Calcium (mg)	852 ± 62	917 ± 66	763 ± 52
Phosphorus (mg)	1290 ± 69	1344 ± 71	1320 ± 64
(CA:P)	1:1.75 ± 0.12	1:1.61 ± 0.07	1:1.93 ± 0.08
Iron (mg)	13.8 ± 0.58	14.2 ± 0.62	15.7 ± 0.66
Sodium (mg)	2572 ± 121	2671 ± 130	2849 ± 139
Potassium (mg)	2385 ± 139	2526 ± 128	2677 ± 126
Vitamin A (IU)	4302 ± 543	4844 ± 538	4940 ± 350
Thiamin (mg)	1.15 ± 0.05	1.16 ± 0.05	1.26 ± 0.05
(mg/1000 Kcal)	0.49 ± 0.01	0.49 ± 0.02	0.50 ± 0.02
Riboflavin (mg)	1.94 ± 0.11	2.09 ± 0.11	1.94 ± 0.09
(mg/1000 Kcal)	0.82 ± 0.03	0.88 ± 0.04	0.76 ± 0.02
Niacin (mg)	20.1 ± 0.84	20.3 ± 0.94	23.1 ± 0.94
(mg/1000 Kcal)	8.71 ± 0.34	8.44 ± 0.24	9.28 ± 0.25
Ascorbic Acid (mg)	58.7 ± 5.6	64.1 ± 5.4	62.0 ± 4.2

¹ Mean ± SEM derived from 17-days of observation in March 1975 and 14-days of observation in June 1976

TABLE 9

NUTRIENT INTAKE PER DINING HALL MEAL
NAVAL AIR STATION, ALAMEDA, CA

BEFORE CASH A LA CARTE (MARCH 1975)

Nutrient	RIK	COM-S	COM-M	ALL
Quantity (gm)	884 ¹	959	756	900
Energy (Kcal)	1171	1254	1018	1188
Protein (gm)	53	57	43	54
Fat (gm)	57	63	52	58
(% Fat Calories)	43.8	45.2	46.0	43.9
Carbohydrate (gm)	114	116	96	114
Fiber (gm)	1.8	1.6	1.4	1.7
Ash (gm)	9.5	9.9	8.2	9.6
Calcium (mg)	690	732	553	697
Phosphorus (mg)	911	960	742	919
(CA:P)	1:1.32	1:1.31	1:1.34	1:1.32
Iron (mg)	7.5	7.6	6.1	7.5
Sodium (mg)	1631	1609	1287	1615
Potassium (mg)	1691	1717	1465	1691
Vitamin A (IU)	2845	3364	2091	2960
Thiamin (mg)	0.69	.70	.62	.69
(mg/1000 Kcal)	0.59	0.56	.61	0.58
Riboflavin (mg)	1.37	1.45	1.09	1.38
(mg/1000 Kcal)	1.17	1.16	1.07	1.16
Niacin (mg)	8.3	8.4	6.5	8.3
(mg/1000 Kcal)	7.1	6.7	6.4	7.0
Ascorbic Acid (mg)	30.6	34.6	35.5	31.8
Dining Hall Meals/Group	564	212	24	800

TABLE 9 (Cont)

AFTER CASH A LA CARTE (JUNE 1976)

Nutrient	RIK	COM-S	COM-M	ALL
Quantity (gm)	759	639	713	699
Energy (Kcal)	1007	827	1002	932
Protein (gm)	46	39	47	43
Fat (gm)	50	42	49	47
(% Fat Calories)	44.7	45.7	44.0	45.3
Carbohydrate (gm)	95	72	94	85
Fiber (gm)	1.3	1.0	1.2	1.2
Ash (gm)	7.3	6.1	7.0	6.7
Calcium (mg)	428	370	335	382
Phosphorus (mg)	573	513	576	549
(CA:P)	1:1.34	1:1.39	1:1.72	1:1.44
Iron (mg)	6.3	5.2	6.8	6.0
Sodium (mg)	1224	1018	1177	1129
Potassium (mg)	1131	974	1021	1041
Vitamin A (IU)	2213	2302	1914	2176
Thiamin (mg)	0.54	0.43	0.51	0.49
(mg/1000 Kcal)	0.54	0.52	0.51	0.52
Riboflavin (mg)	0.92	0.85	0.81	0.86
(mg/1000 Kcal)	0.91	1.03	0.81	0.92
Niacin (mg)	8.2	7.3	9.4	8.14
(mg/1000 Kcal)	8.1	8.8	9.4	8.73
Ascorbic Acid (mg)	27.5	21.7	19.7	23.2
Dining Hall Meals/Group	206	239	143	588

¹ Mean values computed from group rather than individual data

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISSEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H. TERMINATION	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	62760A	3A762760A837		00	020		
B. EXPIRATION							
C. EXPIRATION	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Development of Husbandry Methods for an Outdoor Colony of Macaque Monkeys							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07		76 09		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
EXPIRATION:				76		1.0	
D. NUMBER: Not Applicable				FISCAL		.3	
C. TYPE:				YEAR		23	
E. KIND OF AWARD:				CURRENT		0	
F. CUM. AMT.				77		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Animal Resources Division Department of Comparative Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Morris, J.M., LTC, VC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-2049			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Peace, T., MAJ, VC			
				NAME: Bucci, T.J., LTC, VC POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Laboratory Animals; (U) Primate; (U) Reproduction							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) This project is to study methods for inexpensive domestic outdoor breeding and rearing of nonhuman primates for research. The study is urgently needed to provide a feasible alternative to importation of wild monkeys, a rapidly diminishing resource.</p> <p>24. (U) Four to eight outdoor enclosures will be built at Camp Parks, CA, and populated with macaque monkeys, 90 per enclosure. The animals will be quarantined at LAIR prior to release. Variables to be evaluated include types and distribution of feeders and waterers, shelters, climbing structures, re-introduction techniques, health monitoring and immunization, and catching and restraint methods. Annual reproduction by 80% of breeding females is the goal, with minimal expenditures for labor, equipment, and technical services.</p> <p>25. (U) 76 04-76 09. Eighty-four rhesus monkeys and five cynomolgus monkeys have been obtained as possible breeding stock. These monkeys are being evaluated pending construction of an outdoor facility. The construction has been delayed by CITA (Commercial and Industrial Type Activities) considerations. In compliance with CITA regulations, several contractors interested in conducting this research were located and comparison of costs is in progress.</p>							

^aAvailable to contractors upon originator's approval.

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DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal Resources
Development

WORK UNIT NO. 020

Development of Husbandry
Methods for an Outdoor Colony
of Macaque Monkeys

Rhesus monkeys (Macaca mulatta) are in short supply the world over and, when they are available, the cost is inflationary (it has increased by a factor of 5 during the past 3 years). Cynomolgus monkeys (Macaca fascicularis), which might replace rhesus monkeys in many projects, are presently plentiful, but they face the same pressures that have caused export limitation of the rhesus monkey. The immediate development of a domestic source of rhesus monkeys while breeding stock is available, and collection and evaluation of data on cynomolgus monkeys before their supply is limited, are necessary.

The goal of this work unit is to develop husbandry methods, facilities, equipment, and medical management necessary for efficient, inexpensive domestic production of macaque monkeys. The physiology of the cynomolgus monkey must also be investigated to identify problem areas likely to be encountered in outdoor breeding and to delineate research areas in which it may be interchangeable with the rhesus monkey.

Eighty-four rhesus and five cynomolgus monkeys have been obtained. After quarantine and maturation of young monkeys, regularly cycling females will be identified as potential breeders. Compliance with AR 235-5 is being sought to permit a commercial-industrial "new start" for government-owned, government-operated research in breeding of these essential nonhuman primates.

BODY OF REPORT

WORK UNIT NO. 020

Development of Husbandry
Methods for an Outdoor Colony
of Macaque Monkeys

PROBLEM:

There is a critical shortage of rhesus monkeys (Macaca mulatta) for research because countries to which they are native have curtailed their being exported. The rhesus monkey is used by the U.S. Army for many areas of research. The cynomolgus monkey (Macaca fascicularis) is a possible replacement for the rhesus monkey, at least in some projects. However, the degree of interchangeability of the two species is not known. The cynomolgus faces the same problems that have resulted in scarcity of rhesus monkeys. The Army must provide for its own future needs for these species and must do so in the most expeditious and inexpensive fashion possible. There is also the real danger that no breeding stock will be available after the next 2 to 3 years.

The best solution is to establish domestic breeding colonies of these species. In order to do so, inexpensive, efficient, and scientifically sound methods of husbandry, medical management, and facility design must be developed. In the case of the rhesus monkey, considerable information is available, but little is known about the specifics of outdoor breeding colony operations. The cynomolgus monkey, on the other hand, has seen only limited use in this country as an experimental animal. Little is known of its reproductive biology or climatic adaptability.

The object of this work unit is to develop inexpensive techniques of outdoor colony management, husbandry, and medical care. Problems in facilities design, husbandry methods, disease control and eradication, breeder selection and rearing of off-spring must be solved. For the cynomolgus monkey, ovulatory cycles, hormonal cycles, and social factors beneficial to reproduction must be discovered.

RESULTS AND DISCUSSION OF THE RESULTS:

Eighty-four rhesus monkeys and five cynomolgus monkeys have been obtained. These animals have been quarantined. They are being observed for regular menstrual cycles, maturation, and for behavior patterns.

Environmental Assessment Statements and necessary documentation for MCA funding for construction of the research facility have been filed. Compliance with AR 235-5 is being sought to permit government-owned, government-operated research facilities to be established as the next phase of this research.

CONCLUSIONS:

None.

RECOMMENDATIONS:

Efforts should be continued to obtain documentation to justify a waiver to the provisions of AR 235-5 and thus to obtain authorization to engage in commercial-industrial type activities (research in outdoor breeding of nonhuman primates). In the interim, as many potential breeders as can be accommodated within LAIR should be obtained as they become available, evaluated and held so that they will be available when a domestic breeding study is initiated.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6096	76 09 30	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 04 02	H. TERMINATION	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	672760A	3A762760A837		00		021	
b. EXAMINATION							
c. EXAMINATION							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Studies to Effect Conservation of Monkeys Used for Research							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07		76 09		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER: ^a Not Applicable				FISCAL YEAR		.1	
c. TYPE:				CURRENT		2	
e. KIND OF AWARD:				77		0	
f. CUM. AMT.						0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a				ADDRESS: ^a			
Presidio of San Francisco, CA 94129				Animal Resources Division			
				Department of Comparative Medicine			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Pursue SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Johnsen, D.O., LTC, VC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-2049			
				SOCIAL SECURITY ACCOUNT NUMBER:			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Applicable				NAME: Ward, G.S., MAJ, VC			
				NAME: Bucci, T.J., LTC, VC			
				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Husbandry Research; (U) Primate Clinical Studies; (U) Frimate; (U) Monkey; (U) Laboratory Conditioning; (U) Conservation; (U) Reuse							
23. TECHNICAL OBJECTIVE. ^a 24. APPROACH, 25. PROGRESS (Pursue individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Approximately 3,000 monkeys are currently used annually in USAMRDC, and the countries which export them are curtailing the supply. Alternate means to meet the Army's need for simian primates are imperative. The primary objectives of this study are 1) to establish optimal regimens for laboratory conditioning of newly-imported monkeys so the current 20% loss rate will be minimized and 2) to establish a "recycling center" for Army-owned monkeys which are suitable for reuse.</p> <p>24. (U) A variety of strategies will be used to effect optimal conditioning; monkeys will be received directly from the supplier without his holding them, they will be widely separated during quarantine, and be provided intensive veterinary observation. Animals which become ill will be studied thoroughly to discover the cause of illness and possible predispositions to it. Optimum modes of prophylaxis and therapy will be investigated. Animals for the reuse program will receive similar care upon arrival from their donor; they will be held and a medical history for each will be filed by computer with printouts of the animals inventory available to potential users. Animals available from these studies will be supplied to one or more USAMRDC users.</p> <p>25. (U) 76 04-76 09. This work unit is being terminated due to lack of funds and departure of the principal investigator.</p>							

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal Resources
Development

WORK UNIT NO. 021

Studies to Effect Conserva-
tion of Monkeys Used for
Research

The objective of this work unit was to develop means to conserve non-human primates which are required for Army biomedical research. Their number is dwindling and their cost is multiplying. Conservation was to be effected by two simultaneous approaches: to develop a feasible mechanism by which Army-owned nonhuman primates could be "recycled" for multiple use, and to develop improved medical and husbandry techniques to minimize loss of newly-captured animals. "Recycling" would require a holding facility and excellent documentation of the laboratory experience of each animal so that potential subsequent users could evaluate whether particular animals were appropriate for them. To minimize losses would require large investments in space and veterinary care, as well as quantitative assessment of the many factors which contribute to the stress experienced by monkeys as they adapt to laboratory housing.

This work unit was approved during FY 76, but as important as this objective is, still higher priority was placed on initiating research in domestic breeding of nonhuman primates. Work units were also approved for that purpose and funds for the project area were insufficient for all work units. As a result, no work was accomplished on this work unit. The work unit is being terminated. The information sought can be obtained as part of work to be accomplished under Project 3M76277A812 in the future.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DES'N INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS ^a	9. LEVEL OF SUM ^a
75 07 01	H. TERMINATION	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62760A	3A762760A837		00		022	
B. XXXXXXXXXX							
C. XXXXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Physiologic Aspects of Military Dog Performance							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002600 Biology; 012900 Physiology; 016700 Stress Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 06		76 09		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:		EXPIRATION:		PRECEDING		FUND\$ (in thousands)	
D. NUMBER: Not Applicable				76		2.0	
C. TYPE:		4. AMOUNT:		7T		.5	
E. KIND OF AWARD:		F. CUM. AMT.		77		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Pathology & Comparative Studies Div Department of Comparative Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-4004			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Bucci, T.J., LTC, VC			
				NAME: Geasling, J.W., CPT, VC POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ^a							
(U) Physical Performance; (U) Physiology; (U) Fatigue; (U) Physical Training; (U) Nutrition; (U) Forced Exercise; (U) Stamina; (U) Dogs							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Dogs are used extensively by US Armed Forces--in conjunction with combat operations, for sentry duty at installations of great military and economic value in both CONUS and OCONUS, as sensitive detectors of explosives or contraband and for many other duties which cannot be performed reliably by any other detector system. Although the dogs are rigorously screened prior to selection and trained thereafter, a substantial number of dogs lack stamina. The cause is not understood but appears to be organic rather than psychic. There is a need for detailed information about the factors which influence performance, and how these might be improved by training, diet, selection or other procedures. The object of this study is to develop laboratory and field tests to assess nutritional and physiologic aspects of military dog performance.</p> <p>24. (U) Alterations in total body bioenergetics, intermediary metabolism, nutritional status, cardiopulmonary function and body homeostasis will be studied in dogs during exercise and training and under a variety of environmental conditions. Emphasis will be given to delineation of the factors which limit physical performance, particularly in the German Shepherd, the standard military working dog. Means for improving performance will be sought. Data derived from this work with dogs will be applicable in part to better understanding of the fatigue and physical performance in combat troops as well.</p> <p>25. (U) 75 07-76 09. Progress under this work unit, particularly that dealing with exercise and fatigue in German Shepherd dogs, has been very limited. This is due to delays in completion of Phase 3 of LAIR, delays in procurement and installation of needed equipment and a shortage of technical assistance. Because of these difficulties, this work unit is being terminated.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal Resources
Development

WORK UNIT NO. 022

Physiologic Aspects of
Military Dog Performance

Knowledge of the physiologic aspects of military dog performance would benefit the Army in its use of the dog to accomplish certain security, surveillance, and detection missions. Little progress was achieved in this work unit during the past year, however. The lack of progress stemmed from delay in completion of Phase III, LAIR, delay in procurement and installation of required treadmills, and in shortage of personnel to train the animals and to develop and test the research procedures. These problems are expected to be overcome by the end of the first quarter of FY 77. This work unit is being terminated and certain aspects of the study will be incorporated in a new work unit, WU 003, Project 3M762772A812.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISB'N INSTN ^a	9. SPECIFIC DATA CONTRACTOR ACCESS ^a	10. LEVEL OF SUM A. WORK UNIT
76 03	H. TERMINATION	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A837	00	023			
b. CONTRACTING							
c. EXHIBITION	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Ascorbic Acid and Its Effects on Bordetella bronchiseptica Infection in Guinea Pigs							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
012900 Physiology; 002600 Biology; 002300 Biochemistry							
13. START DATE	14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD		
76 03	76 09		DA		C In-House		
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
d. NUMBER: ^a Not Applicable				76		.2	
c. TYPE:				FISCAL		.1	
e. KIND OF AWARD:				YEAR		0	
f. CUM. AMT.				77		0	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a				ADDRESS: ^a			
Presidio of San Francisco, CA 94129				Animal Resources Division Department of Comparative Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Peace, T., MAJ, VC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-2049			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME: Shroyer, E., MAJ, VC			
				NAME:			
				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Military Susceptibility to Infections; (U) Infection; (U) Bordetella bronchiseptica; (U) Ascorbic Acid; (U) Guinea Pigs							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Guinea pigs are an important resource in medical research. Ascorbic acid is essential in their diet. Approximately 10% of guinea pigs are lost each year to bacterial pneumonia; infection with <u>B. bronchiseptica</u> is a common cause. The erratic susceptibility of these valuable animals (\$8.00 ea for adults; \$50.00 ea pregnant females) may be related to variations in dietary ascorbic acid. This study will establish quantitatively whether the susceptibility of guinea pigs to <u>B. bronchiseptica</u> is affected by the amount of dietary ascorbic acid. The results will also introduce quantitative considerations into the relationship between ascorbic acid nutrition and susceptibility to infection in soldiers.</p> <p>24. (U) The aerosol concentration of <u>B. bronchiseptica</u> sufficient to produce pulmonary infection in specific-pathogen-free (SPF) guinea pigs will be determined. Groups of SPF guinea pigs will be fed diets containing controlled amounts of ascorbic acid --- deficient, normal, and excessive. The guinea pigs will then be exposed to the <u>B. bronchiseptica</u> challenge, and the number of organisms recoverable from their lungs on the 14th day after exposure will be determined. This number and the degree of pneumonia will serve as a measure of the susceptibility of each group, as a function of dietary ascorbic acid.</p> <p>25. (U) 76 03-76 09. Five of seven objectives of the pilot study have been accomplished satisfactorily. It is planned to satisfy the remaining objectives of the pilot study and proceed with the definitive study. This work will be continued in Project Area 3M76277A812 in FY 77.</p>							

^a Available to contractors upon originator's approval.

407

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal Resources
Development

WORK UNIT NO. 023

Ascorbic Acid and Its
Effects on Bordetella
bronchiseptica Infection
in Guinea Pigs

Bordetella bronchiseptica can be isolated from the respiratory tract of apparently healthy guinea pigs in conventional colonies. Although frequently associated with pneumonia and other respiratory tract infections, it is usually considered to be an opportunistic organism. Partially depending upon such stress factors as malnutrition, crowding, prolonged temperature extremes, pregnancy, and experimental manipulations, the animals may either develop a carrier state or overt signs of illness. A lowered resistance of the animal may upset the balance of the carrier state.

Evidence of a correlation between ascorbic acid deficiency and lowered resistance to bacterial infection has been reported. It is possible that suboptimal dietary intake of ascorbic acid by guinea pigs is one factor which contributes to the puzzling sporadic outbreaks of bacterial disease that occur in apparently stable guinea pig colonies.

This study is expected to provide data on the relationship between ascorbic acid intake and susceptibility to infection by B. bronchiseptica. The primary objective is to determine whether ascorbic acid doses of 0.25mg/100g body weight/day (low), 2.50mg/100g b.w./day (adequate), and 18mg/100g b.w./day (high) are responsible for significant differences in the susceptibility of various groups of guinea pigs exposed to standard challenge doses.

BODY OF REPORT

WORK UNIT NO. 023

Ascorbic Acid and Its
Effects on Bordetella
bronchiseptica Infection
in Guinea Pigs

PROBLEM:

Guinea pigs are among the most important laboratory animals. In 1974, more than 600,000 were used for research in the United States; approximately 3,700 were used at LAIR in FY 75.

Sporadic disease in guinea pigs is a major cause of animal deaths and of wasted research resources. At LAIR, 15% of 108 spontaneous deaths during CY 1975 were caused by bacterial pneumonia. To define and minimize this waste of resources, this study was undertaken to provide objective data about the specific interaction between dietary ascorbic acid and infection by Bordetella bronchiseptica in guinea pigs. Evidence of a correlation between ascorbic acid deficiency and lowered resistance to bacterial infection has been proposed, but conflicting results have also been reported. These controversies may be due to variables that are not controlled properly. Hence, this investigation will be based upon quantifiable considerations of bacterial exposure and dietary ascorbic acid intake by guinea pigs housed in a defined environment.

RESULTS AND DISCUSSION OF THE RESULTS:

B. bronchiseptica has been aerosolized without a significant loss of viability during and following aerosolization, without differences in the viability of the aerosol from experiment to experiment, and without significant variations in the distribution of organisms within the aerosol cloud.

An intranasal inoculum of 10^4 organisms produced infection in all guinea pigs and resulted in antibody titers between 1:20 and 1:80 fourteen days after infection.

The B. bronchiseptica isolated from experimentally infected lung and trachea has been quantified by plating tissue suspensions on modified MacConkey agar and subsequent counting of pure cultures.

The normal pulmonary flora of the (crl: COBS (HA)) guinea pigs has been identified. Two groups of guinea pigs have been maintained within the LAIR barrier system for up to 15 days without bacterial contamination from the surrounding environment.

The only tasks that remain to be accomplished during the pilot study are to establish chronic ascorbic acid deficiency in the guinea pigs and to determine the optimal population of B. bronchiseptica that will result in a latent infection of the guinea pigs. These objectives will be accomplished when additional guinea pigs are available.

CONCLUSIONS:

None.

RECOMMENDATIONS:

It is recommended that upon satisfactory completion of pilot study, work be initiated on the definitive study.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
76 04 02	H. TERMINATION	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A837	00	024			
b. CONTRACTOR							
c. XXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Study of Chronic or Recurrent Diseases of the Military Working Dog							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine; 002600 Biology; 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		76 09		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER: Not Applicable				76		.4	
c. TYPE:				7T		.1	
e. KIND OF AWARD:				CURRENT		0	
f. CUM. AMT.				77		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Pathology & Comparative Studies Div Department of Comparative Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Applicable				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Panosteitis; (U) Dogs; (U) German Shepherd Dogs; (U) Military Working Dog							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Dogs are used extensively by US Armed Forces. This study is designed to investigate the chronic and recurrent diseases which impair the effectiveness of the dogs, and to seek means to ameliorate or eliminate the diseases. One such disease is panosteitis, a syndrome characterized by transient, recurrent lameness in young dogs. Radiographically it is a medullary density in the shaft of long bones of the limbs. The pathogenesis and etiology of panosteitis are unknown. It occurs most frequently in German Shepherd dogs, the breed used as a military working dog and recently selectively bred to better fulfill military requirements. An episode of panosteitis debilitates a dog for several weeks.</p> <p>24. (U) The study is to clarify the pathogenesis and cause of the disease, with the hypothesis that it is infectious and conditioned by heredity. Dogs with clinical and radiographic signs of panosteitis were identified. ⁹⁹Technetium bone scanning was evaluated. Biopsies were studied by light microscopy. Transmission to kidney cell cultures and to young pups will be attempted using biopsy material and blood.</p> <p>25. (U) 76 04-76 09. Three dogs developed panosteitis. Biopsy material and blood from one dog were placed in tissue culture. No cytopathic effects were observed in several autologous and heterologous cell lines. Delayed hypersensitivity skin tests on two dogs were also negative. The biopsies yielded tissue unsuitable for histologic evaluation. Radioactive technetium scans were continued and refined. Isotope scanning revealed lesions 7-14 days earlier than did x-ray. Increased doses of radioactive material provided only a limited improvement in resolution. Radiographs remain the best method for clinical interpretation and diagnosis of panosteitis lesions. Work Unit is being terminated due to lack of funds.</p>							

^aAvailable to contractors upon originator's approval.

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DD FORM 1498
1 MAR 66

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal Resources
Development

WORK UNIT NO. 024

Study of Chronic or Recurrent
Diseases of the Military Work-
ing Dog

The following investigation has been conducted under this Work Unit:

STUDY #1 Study of the Etiology, Biological Parameters,
and Control of Panosteitis of Dogs

Panosteitis is a syndrome characterized clinically by debilitating, transient, and recurrent lameness in young dogs. It is usually accompanied by fever, occasionally by eosinophilia. Radiographically, fully developed lesions appear as densities in the marrow canal of long bones. The German Shepherd is the breed most frequently affected; the disease has occurred in dogs produced in the Army's Biological Sensor Research Program. While the strong breed predilection has suggested a heritable cause for the condition, its cause is unknown. Elucidation of the cause may afford the opportunity for control of the condition.

Several dogs from affected bloodlines were regularly exercised on a treadmill in order to precipitate clinical signs of panosteitis. Those which developed lameness were monitored with weekly radiographs and physical examinations. Of the lamenesses that developed, only three were attributed to panosteitis. Subsequent studies involved these dogs.

Gamma scans, using ⁹⁹Technetium diphosphate, demonstrated increased medullary activity seven to fourteen days prior to the appearance of radiographically-demonstrable lesions. Bone biopsies and blood from affected dogs were used for cell cultures, delayed hypersensitivity tests, and histological evaluation. The results of these procedures did not reveal the cause of the disease.

BODY OF REPORT

WORK UNIT NO. 024

Study of Chronic or Recurrent Diseases of the Military Working Dog

STUDY NO. 1

Study of the Etiology, Biological Parameters, Pathogenesis and Control of Panosteitis of Dogs

PROBLEM:

The cause and course of panosteitis are unknown. It occurs most frequently in the German Shepherd, a breed used as military working dogs. The disease is characterized by pain and lameness in one or more limbs, usually beginning in one and proceeding to others over a course of one to several weeks. The syndrome includes intermittent fever, inconstant eosinophilia, and radiographic and histologic evidence in involved bones of diffuse endostial mineralization with subsequent resolution. Upon resolution, some dogs remain healthy while others "recycle" with the disease weeks or months later. The association of the syndrome with this one breed is suggestive of peculiar genetic susceptibility, but its occurrence almost exclusively in young dogs, its cyclic nature, and rapid resolution also suggest an infectious process. The objective of this study is to define the nature of this disease, to discover its cause if possible, and to develop control measures.

During this reporting period, animals were exercised on a treadmill in an effort to make subtle lamenesses more evident. Each dog was exercised daily. Those showing lameness were further evaluated by physical and radiographic examination. Biopsies taken from affected bones as well as "acute" blood samples were used in an attempt to induce pathologic changes in cell cultures. Some biopsies were evaluated histologically and others were used for delayed hypersensitivity skin tests.

RESULTS AND DISCUSSION OF THE RESULTS:

Although lameness was detected in several dogs, a positive diagnosis of panosteitis was made in only three animals.

Sequential scans using 40 mCi ⁹⁹Tc Technetium disphosphate revealed progression of the disease in affected bones. Blood and biopsy material from one affected animal was used to inoculate autologous monocyte cultures, and plasma filtrates were inoculated into canine, feline, and vervet monkey kidney cell lines. Cytopathic effects were not produced in any of these tissue culture systems. Weekly radiographic examination was used to monitor the course of the disease. Multiple half-thickness bone biopsies and marrow aspirates were obtained from radiographically

positive and gamma scan positive locations. Biopsies and aspirates were obtained from corresponding locations on the unaffected opposite leg to provide control tissue. Interpretation of histologic sections of bone biopsies was not possible because of artifacts produced during tissue collection. Following sensitization with dinitrochlorobenzene (DNCB), multiple skin tests using biopsy material and aspirate were performed. Only the DNCB control sites reacted positively with delayed hypersensitivity.

Skin tests were performed during the active phase of the disease in a second affected dog. Hypersensitivity was not demonstrable.

CONCLUSIONS:

⁹⁹Techneium scans demonstrate active foci of panosteitis seven to fourteen days before they can be visualized radiographically, but offer far less resolution than conventional radiography. The value of gamma scans lies in their ability to demonstrate the disease in the acute phase prior to the time lesions are radiographically evident. Identification of these early lesions thus provides access to them and should enhance the likelihood of discovering the cause.

The inability of blood and biopsy material to cause cytopathic effect in tissue culture coupled with the failure to demonstrate a foreign antigen with delayed hypersensitivity skin tests suggests that the cause is not an infectious agent.

Two of the three dogs that developed panosteitis were siblings of previously affected dogs, which reaffirms the hereditary predisposition.

Nearly all cases of panosteitis involve bones of the forelimb. In many dogs, radiographic evidence of the disease is limited to this location. This distribution may be related to a greater percentage of the animals' weight being borne by the forelegs.

RECOMMENDATIONS:

Because of the high cost of maintaining test animals, the relatively low incidence of active disease, and the lack of tangible results, this study should be terminated. The dogs, especially those with a history of panosteitis, should be retained for use in other chronic studies. Limited research might then be undertaken when an exacerbation is discovered.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY						1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
						DA OE 6088	76 09 30	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTRN	8B. SPECIFIC DATA- CONTRACTOR ACCESS		9. LEVEL OF SUM	
75 07 01	H. TERMINATION	U	U	N/A	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT	
10. NO./CODES*		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		672760A		3A762760A837		00		025	
b. XXXXXXXX									
c. XXXXXXXX		CARDS 114 (F)							
11. TITLE (Precede with Security Classification Code)*									
(U) Basic Studies in Reproduction of Owl Monkeys (Aotus species)									
12. SCIENTIFIC AND TECHNOLOGICAL AREAS*									
002600 Biology; 012900 Physiology; 005900 Environmental Biology									
13. START DATE			14. ESTIMATED COMPLETION DATE			15. FUNDING AGENCY		16. PERFORMANCE METHOD	
75 07			76 09			DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS		b. FUNDS (In thousands)	
a. DATES/EFFECTIVE:				EXPIRATION:		PREVIOUS		76	
b. NUMBER: Not Applicable						FISCAL		77	
c. TYPE:				d. AMOUNT:		CURRENT		.3	
e. KIND OF AWARD:				f. CUM. AMT.		77		0	
19. RESPONSIBLE DOD ORGANIZATION					20. PERFORMING ORGANIZATION				
NAME: Letterman Army Institute of Research					NAME: Letterman Army Institute of Research				
ADDRESS: Presidio of San Francisco, CA 94129					ADDRESS: Animal Resources Division Department of Comparative Medicine Presidio of San Francisco, CA 94129				
RESPONSIBLE INDIVIDUAL					PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)				
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21. GENERAL USE					SOCIAL SECURITY ACCOUNT NUMBER:				
Foreign Intelligence Not Applicable					ASSOCIATE INVESTIGATORS				
					NAME: Morris, J.M., LTC, VC				
					NAME: Bucci, T.J., LTC, VC POC:DA				
22. KEYWORDS (Precede EACH with Security Classification Code)									
(U) Primate; (U) Reproduction; (U) Owl Monkey; (U) Aotus; (U) Estrus; (U) Primate Behavior									
23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
<p>23. (U) A source of owl monkeys is crucial to the Army's malaria research program. Several hundred are used each year in development of antimalarial drugs, in maintaining a supply of the malaria parasite for study, and for immunologic studies necessary to develop a vaccine. No other test system is available for this work. The availability of wild animals captured in South America is very uncertain. A successful domestic breeding program would insure a supply of these animals, but virtually nothing is known of their reproductive physiology. The object of these studies is to determine the physiologic characteristics and husbandry principles necessary to establish a successful breeding colony.</p> <p>24. (U) Adult owl monkeys will be karyotyped and housed in pairs in suspended wire cages having wooden nest boxes. Illumination will be controlled in a reversed day/night cycle. Reproductive behavior and other social interactions will be observed both directly and remotely with a low-light-sensitive video camera. Handling of animals will be minimized. Determination of estrus cycle and diagnosis of pregnancy will be determined by biochemical assay, palpation, and observation of changes in behavior and conformation. Spermatogenesis will be determined by electroejaculation and needle biopsy.</p> <p>25. (U) 75 07-76 09. Eight live baby owl monkeys have been born to 14 pairs. Economical and efficient caging systems have been developed. Proper room temperature, humidity and lighting have been determined. Characterization of behavior has begun.</p>									

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1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68
AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal
Resources Development

WORK UNIT NO. 025

Basic Studies in
Reproduction of Owl
Monkeys

Owl monkeys (*Aotus* species) are essential to the Army's malaria research program and they are no longer available in sufficient number from OCONUS sources. Domestic breeding programs are imperative to maintain an adequate supply of these animals. To date, attempts to breed owl monkeys in captivity have not been successful consistently.

The goal of this work unit is to develop basic information about the reproductive biology and husbandry techniques for maintenance of a successful breeding colony of *Aotus* monkeys.

A total of 45 adult animals are presently in the colony. Of these, 32 are paired, with only 7 optimally matched karyotypic pairs. Eight live babies have been produced in the first year of operation. Further studies are planned on owl monkey behavior, nutrition, and reproductive endocrinology. Additional owl monkeys are being sought.

BODY OF REPORT

WORK UNIT NO. 025

Basic Studies in Reproduction of Owl Monkeys

PROBLEM:

There is a critical shortage of owl monkeys (*Aotus* species) for biomedical research since the countries of origin have severely limited exportation. Owl monkeys are the only subhuman primates in which infections with human falciparum malaria can be maintained. The Army is dependent upon these monkeys for malaria research. Continued availability of these animals is essential for anti-malarial drug testing, for studies of the pathogenesis of the disease, and for maintaining a supply of the malarial parasites.

The long-term solution to provide a source of these animals is a domestic breeding program. Little is known about the reproductive biology of owl monkeys; and, to date, breeding these animals in captivity has not been successful consistently.

The objective of this study is to determine the environmental and husbandry conditions conducive to successful laboratory breeding and rearing of owl monkeys. Involved are studies to determine optimal caging arrangements, light cycles, and diet, and to develop economical, labor-efficient cage facilities and husbandry procedures.

Related studies include characterization of normal and abnormal behavior, diagnosis of pregnancy, determination of ovulatory cycles, length of gestation, and periods of spermatogenesis.

Experience in other laboratories has revealed that these monogamous, nocturnal animals are extremely sensitive to interruptions in routine, susceptible to infectious disease, and that random pairing of animals results in a high incidence of social incompatibility or infertile matings. At least seven different karyotypes have been found in this genus with four recognizable phenotypes. Some karyotypes have much lower fertility rates than others. Since monkeys with the same phenotype may have different karyotypes, cytogenic analysis is essential prior to pairing.

RESULTS AND DISCUSSION OF THE RESULTS:

Initially, thirty-five monkeys were obtained from the US Army Institute of Infectious Diseases, Ft Detrick, MD, on 14 May 1975. These animals were matched by body weight and caged in pairs in standard two-tiered monkey cages. Three males and one female were caged individually. One male from this group later died from a ruptured aortic aneurysm.

Owl monkeys originate in sub-tropical regions; to mimic these conditions, the temperature is kept at 26.7° C and the humidity at 65%. These environmental conditions appear vital to the health of the colony, since mild upper respiratory disease occurs when the conditions are temporarily altered. Owl monkeys are omnivorous in the wild with much of their diet containing non-vegetable material. The owl monkeys are fed twice daily on a varied diet including a commercial high protein monkey chow soaked in orange-flavored drink, orange juice, bananas, vitamins and minerals, high protein baby cereal, eggs, a commercial marmoset diet, oranges, apples, and sweet potatoes.

Since owl monkeys are nocturnal, they are maintained with a reversed light/dark cycle. The daylight cycle, provided with normal fluorescent lights, is from 2100 hours to 0830 hours. The night cycle, or dark cycle, is maintained with reduced red light from about 0900 hours to 2030 hours. There is a staged sunrise and sunset lasting 1/2 hour.

Cytogenetic analysis of the first group of owl monkeys was performed by Dr. T.C. Jones of Pathobiology Inc., Marlboro, MA, in September 1975. Seven females and seven males are Type II ($2n=54$), nine females and nine males are Type III ($2n=53$), and two males are Type IV ($2n=52$).

Theory and preliminary experience at New England Primate Center indicate that the Type II and Type IV pairs have the best chance to produce viable offspring. Type III pairs or pairs of mixed type have only marginal chances for success.

On 7 October 1975 the animals were paired by karyotype and temperament. This pairing resulted in seven Type II pairs, seven Type III pairs, two Type II and Type IV pairs, and two Type III unpaired males. The first offspring from these animals was born on 31 December 1975. By September 1976, eight live births and two abortions had occurred. Five of the live babies are from Type II parents. One live baby is from a Type III X Type IV cross. One Type II pair has produced two live babies, 7 months apart. Type III matings have resulted in one live birth and two abortions. This experience emphasizes the importance of pairing breeders by karyotype.

Attempts to diagnose pregnancy in owl monkeys have been marginally successful. About four to six weeks prior to parturition, enlargement of the upper thoracic region has been noted. Owl monkey mammary glands are located in the axillary region. The air sacks in the neck appear enlarged as well. Abdominal palpation for pregnancy has been attempted on three animals. A hard round mass, approximately 2.5 cm in diameter, in mid-abdomen was palpated. Each animal gave birth to a live baby six weeks later.

The original standard monkey cages were replaced by one of two modified cages. One was a horizontal baboon cage, 100 X 75 X 90 cm. The other was a vertical cage made by welding two standard monkey cages together.

These cages have been in use for ten months and successful births have occurred in both types. The monkeys are more active and easier to observe in the horizontal cage. Both cages use a waste pan which empties into a gutter. The disadvantages of both cages are that they are difficult to clean and they do not retain all the waste. Consequently, extensive floor cleaning is required. These cages cost about \$1,000 each.

An improved cage system has been designed and constructed by laboratory personnel using 14 gauge 1 X 1 inch welded wire. The cage is 75 X 125 X 90 cm and is hung from overhead supports. The cage bottom is 126 cm from the floor. Waste is allowed to fall onto the floor and is flushed to drains. The floor is free of all obstructions. The cages have several advantages: they are very economical to construct (about \$12 each), they are easy to clean, the animals are easy to observe, and the animals are more active and consume more food than in any of the other cages.

In nature, owl monkeys use nesting holes. With their natural habitat in mind, a nest box of marine plywood with a wire bottom was designed. The box is 30 X 30 X 45 cm with a 15 cm entrance hole and a removable top for access to animals and cleaning. The boxes are hung outside the wire cages. They are used also to catch animals since they are a place of retreat when the animal is alarmed. The box can serve as a temporary metabolism cage.

Three additional groups of owl monkeys have been added. Three animals (two males and one female) were received from Florida State University on 8 March 1976. Five animals (3 females and 2 males) were received from Yale University on 30 June 1976. Three animals (a pair with a juvenile offspring) were received from Delta Regional Primate Research Center. Phenotypically, these owl monkeys appear to be different than those received from Ft Detrick in May 1975. These newly received animals have not yet been karyotyped.

Several experiments are planned to investigate owl monkey reproductive biology. Urine can now be obtained from nest boxes without disturbing the animals. Urine will be used for routine urinalysis, determination of urinary estrogens, and possibly for early pregnancy diagnosis by demonstration of chorionic gonadotropin. The presence of "pregnancy zone protein" in a small serum sample may reveal pregnancy. The owl monkey's caloric requirement will be assessed by measuring food intake during various physiological states.

CONCLUSIONS:

None.

RECOMMENDATIONS:

The study should be continued as planned.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OE 6108	76 10 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
76 05 06	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M762772A812				004	
b. SECONDARY	62760A	3A762760A837				026	
c. THIRDARY	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Animal Models for Surgical Repair of Musculoskeletal Structures							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002600 Biology; 003500 Clinical Medicine; 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 05		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
d. NUMBER ^a Not Applicable				76		.3	
e. TYPE:				FISCAL		.1	
f. CUM. AMT.				77		2.5	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research				NAME ^a Letterman Army Institute of Research			
ADDRESS ^a				ADDRESS ^a			
Presidio of San Francisco, CA 94129				Department of Comparative Medicine			
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NAME: Canham, J.E., COL, MC				Presidio of San Francisco, CA 94129			
TELEPHONE: (415) 561-3600				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
21. GENERAL USE				NAME ^a Rodkey, William G., CPT, VC			
Foreign Intelligence Not Applicable				TELEPHONE: (415) 561-3760			
				SOCIAL SECURITY ACCOUNT NUMBER:			
				ASSOCIATE INVESTIGATORS			
				NAME: Geasling, Jay W., CPT, VC			
				NAME: Lollini, Lance O., MAJ, VC POC:DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Surgical Repair; (U) Extensor Tendon; (U) Nerve; (U) Muscle Transplantation; (U) Trauma; (U) Nerve Graft; (U) Microsurgical Technique							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Combat and non-combat injuries to military personnel very frequently affect extremities and many result in massive loss of tissue. Repair and rehabilitation are extremely costly in medical resources, lost duty days and permanent patient disability. To minimize debility, disability and amputations and to conserve the fighting strength through maximum restoration of function, pioneering efforts are required to advance the current level of surgical treatment of these patients. These studies will provide experimental data on which improved techniques of surgical and microsurgical reconstruction and post-surgical rehabilitation may be based.</p> <p>24. (U) Appropriate animal models will be developed to study injuries of soldiers which are serious and numerous but for which current restorative and reconstructive techniques are inadequate or controversial. Traumatized nerves will be repaired using interfascicular grafts or by an epineurial technique under tension, and these will be evaluated to determine which technique provides greatest return of function. Extensor tendons, severed in the metacarpal region, will be reconstructed, and an optimum post-operative immobilization period will be determined by maximum return of function. Selected tendons will be examined microscopically to evaluate cellular events involved with extensor tendon healing. Subsequent to trauma and loss of muscle mass, skeletal muscles will be autotransplanted or free grafted to the site of destruction. Restoration of limb function will be evaluated.</p> <p>25. (U) 76 05-76 09. Work has begun under this work unit, and suitable animal models have been identified. Some of the initial surgical procedures have been performed, and evaluations will begin soon.</p>							

^aAvailable to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A762760A837

Military Animal Resources
Development

WORK UNIT NO. 026

Animal Models for Surgical
Repair of Musculoskeletal
Structures

The following studies were initiated under this Work Unit during the past year:

- | | |
|----------|--|
| STUDY #1 | Nerve Repair in Cats: Grafts vs. Tension |
| STUDY #2 | Digital Extensor Tendon Repair in Monkeys |
| STUDY #3 | Study of Autogenous Graft Techniques to Restore Muscle Function After Traumatic Injury |

STUDY #1. The proximal ulnar nerve of the domestic cat is a satisfactory model for repairs of peripheral nerve injuries. Five animals have undergone initial repair by each of the two techniques. Evaluation and comparison of the outcome will begin in six months.

STUDY #2. Information is lacking relative to healing of extensor tendons. Using the common digital extensor tendons of the rhesus monkey, this study will determine the optimal period for immobilization of a repaired tendon, and will identify the cellular processes involved in healing. Twenty-four rhesus monkeys have undergone transection and initial repair of common digital extensor tendons. Surgical biopsies of the repair sites are scheduled.

STUDY #3. Adequate techniques for restoring massively traumatized muscle currently do not exist. This study will develop techniques in animals which will permit restoration of function to large skeletal muscles that have been severely traumatized. The first three free muscle grafts have been performed in domestic cats. One cat has been studied electromyographically; it has definite voluntary function in at least one of two grafted muscles.

BODY OF REPORT

WORK UNIT NO. 026

Animal Models for Surgical
Repair of Musculoskeletal
Structures

STUDY #1

Nerve Repair in Cats, Grafts
vs. Tension

PROBLEM:

Disabling wounds of upper and lower extremities accounted for 54% of all casualties in military hospitals during one 24-month period of the Vietnam conflict. Many of these injuries included severe damage to peripheral nerves. Advances have been made during the past decade in the surgical repair of peripheral nerves, but the results are still far from satisfactory. Controversy exists especially about the preferred method of surgical repair of peripheral nerve injuries when a substantial gap exists between the ends of a severed nerve. It is believed that tension at the repair site is one of the most important detrimental local factors to the nerve regeneration process. The question arises, therefore, whether to mobilize and stretch the nerve for an end-to-end repair or whether to use nerve grafts, or a single graft, and thus eliminate tension at the repair site. Furthermore, there is a paucity of data on objective evaluations comparing nerve grafts without tension with nerve repairs with tension. Nearly all evaluations of both clinical data and experimental results have been purely subjective. This study is designed to provide a critical comparison by objective evaluation of epineurial end-to-end repairs with tension vs. interfascicular nerve grafts without tension.

RESULTS AND DISCUSSION OF THE RESULTS:

Work has recently begun on this study. The proximal ulnar nerve of the domestic cat has been identified as a satisfactory model for studying the techniques of peripheral nerve repairs. Thus far, five animals have undergone initial surgical repair of both proximal ulnar nerves, one nerve being repaired end-to-end under tension and the other nerve being repaired with interfascicular grafts without tension. These initial microsurgical nerve repairs have been completed with a minimum of technical problems. Evaluation of the nerve repairs and comparison of return of function will begin within six months.

CONCLUSIONS:

None.

RECOMMENDATIONS:

Each of the two surgical techniques will be performed in a total of 20 cats. Five to six months following the nerve repairs, cats should be observed subjectively for ambulation, sensation, and use of intrinsic muscles. Critical objective evaluations should include muscle efficiency and maximum muscle contraction of the reinnervated muscles. Weights of reinnervated muscles should also be compared. Total axon counts should be made proximal and distal to nerve repair sites as well as from the center of nerve grafts. All accumulated data should be evaluated by appropriate statistical analyses.

PUBLICATIONS:

None.

STUDY #2

Digital Extensor Tendon Repair
in Monkeys

PROBLEM:

Extensor tendon injuries occur frequently in both combat and non-combat situations. The study of tendon injuries and their repair has centered primarily around flexor tendons. Great controversy exists regarding the optimum duration of immobilization following the repair of an injured extensor tendon. Commonly, an injured extensor tendon is fully immobilized in plaster for four weeks following a repair. However, surgeons have reported many serious difficulties with this length of immobilization. Prolonged immobilization of a repaired extensor tendon results in tenodesis. This leads to either a permanent disability of the extremity, or it may require additional surgical procedures such as tenolysis to restore mobility to the tendon. Conversely, using a repaired extensor tendon too early will result in repair failure or extensor lag. This also leads either to a permanent disability or to repeated surgical procedures to correct these problems. The cellular processes involved in healing of extensor tendons have not been studied histologically and considerable controversy exists over the healing processes of tendons generally. This study will help to determine the optimal period of immobilization of a repaired extensor tendon. Additionally, studies will be done to identify the cellular processes involved in healing of digital extensor tendons repaired after transection in the metacarpal region.

RESULTS AND DISCUSSION OF THE RESULTS:

Work recently has been initiated on this study. Twenty-four rhesus monkeys have undergone transection and initial repair of extensor tendons in one hand. At designated times, the repaired tendons will be allowed limited range of motion. Goniometer measurements will be made before

surgery and throughout the recuperative period to determine the return of mobility to the operated hand. Biopsies of repaired tendons will be taken at 10 weeks after the repair.

CONCLUSIONS:

None.

RECOMMENDATIONS:

This study should be continued within the scope of its original plan. Rhesus monkeys are uniquely suitable as models for this study of digital extensor tendons because each digit is supplied with two extensors, the common and the underlying proprius. The tendons to the index and the small fingers can be severed and repaired, and the repair site subsequently removed for analysis. At that time, the extensor function can be restored to those digits by union of the proprius extensor tendon from adjacent fingers with the distal stumps of the common digital extensors in the injured fingers. Thus, the monkey will be restored to full extensor tendon function and will experience no disability.

PUBLICATIONS:

None.

STUDY #3

Study of Autogenous Graft
Techniques to Restore Muscle
Function After Traumatic
Injury

PROBLEM:

During the last three wars in which US troops were engaged, the percentage of debilitating musculoskeletal injuries of the upper and lower extremities has progressively increased. Many of these injuries have resulted in massive loss of skeletal muscle tissue. Additionally, the currently accepted technique for treatment of severe wounds to skeletal muscles includes extensive debridement of all devitalized tissues. Such surgical treatment often leads to disfigurement and loss of function. Techniques for surgically restoring or augmenting such destroyed muscle tissue are nearly nonexistent. Neither free grafts nor muscle autotransplantation have been used successfully to augment or replace large muscle masses which have been severely traumatized. This investigation will attempt to develop techniques in animals which will permit restoration of function to large skeletal muscles that have been severely traumatized or destroyed. Studies will include both free muscle grafts and translocation of large muscle masses by using microsurgical neurovascular techniques.

RESULTS AND DISCUSSION OF THE RESULTS:

Work on this study has just commenced. Three cats have undergone free muscle grafts. At this time, only one cat has been studied electromyographically. In this animal, it was found that one of the free muscle grafts is viable and shows an indication of early reinnervation.

Additional surgical procedures are planned and all grafted muscles will be studied electromyographically at the designated time.

CONCLUSIONS:

None.

RECOMMENDATIONS:

Based on the positive results observed in the first experimental animal, this study will continue as originally planned. Attempts will be made to determine the necessity for denervating a muscle prior to using it as a free muscle graft. All grafted and transplanted muscles should be studied by electrodiagnostic means as well as by histochemical and histopathological techniques. If additional success is obtained in free grafting small muscles, efforts should be concentrated on moving larger and larger skeletal muscles, or by reconstructing a large mass with successive small ones. When adequate techniques for muscle restoration have been developed in the cat, these techniques should then be applied to a number of nonhuman primates.

PUBLICATIONS:

None.

APPENDIX A

PUBLICATIONS - FISCAL YEARS 1976 AND 1977

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27. FOWLER, J. L., D. L. STUTZMAN, J. F. FOSTER AND W. H. LANGLEY. Report of 1973 microbiological data collection program. November 1975.
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- 76-012 KING, R. D., H. A. KHAN, J. C. FOYE, J. H. GREENBERG AND H. E. JONES. Transferrin, iron, and dermatophytes. I. Serum dermatophyte inhibitory component definitively identified as unsaturated transferrin. *J. Lab. Clin. Med.* 86: 204, 1975.
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- 76-014 HUSTON, R. L., P. C. WEISER, G. L. DOHM, E. W. ASKEW AND J. B. BOYD. Effects of training, exercise and diet on muscle glycolysis and liver gluconeogenesis. *Life Sci.* 17: 369, 1975.
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- 76-033 LUFKIN, E. G., O. D. TAUNTON, F. B. STIFEL, F. D. HOFELDT, M. R. WRENSCH, L. HAGLER AND R. H. HERMAN. Effect of Tri-iodothyronine on human jejunal glycolytic enzymes (39046). Proc. Soc. Exp. Biol. Med. 150: 410, 1975.
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APPENDIX B

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